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A SELECTIVE INDICATOR MEDIUM FOR ISOLATING STREPTOMYCES SCABIES FROM POTATO TUBERS OR SOIL[1]

J. D. Menzies and Caroline E. Dade[2]

SUMMARY

An agar medium containing 2.5% sodium caseinate, 1% $NaNO_3$, and 0.1% tyrosine is recommended for isolating *Streptomyces scabies* from potato scab lesions or from soil. This medium inhibits spreading bacteria and favors the production of a dark brown pigment closely encircling colonies of the pathogen. Almost all pathogenic isolates of *S. scabies* produce pigment, but most nonpathogenic actinomycetes do not. This reaction aids in the selection of the probable pathogen from dilution plates containing other morphologically similar actinomycetes.

Streptomyces scabies, (Thaxt.) Waksman & Henrici, the actinomycete causing scab in potatoes, is very difficult to distinguish in culture from many of the saprophytic actinomycetes abundant in soil and on tubers. In isolating this organism from superficial scab lesions, or especially from soil, it is therefore necessary to verify the pathogen by pathogenicity tests. For the purpose of reducing the number of isolates requiring verification, a tyrosine-casein-nitrate medium was developed that selects probable *S. scabies* isolates on the basis of their ability to produce a brown to black melanin-type pigment.

Taylor and Decker (8) found that pathogenic strains of *S. scabies* produced a "brown ring" reaction in skim-milk tubes. Others later verified this correlation (3, 9), and showed that the melanin-like pigment depends on the presence of tyrosine in the substrate (1, 4, 6, 9). It is generally agreed that, although some saprophytic actinomycetes also produce this pigment, few verified isolates of *S. scabies* fail to do so. The probable pathogen can therefore be selected preliminarily by taking pigment-producing actinomycete colonies from dilution plates. If a surface-sterilization and trituration method such as Taylor's (7) is used in isolating from scab lesions, the contamination by other actinomycetes is so low that brown-pigment types almost certainly are the pathogen. Isolations from soil are much more difficult because of the high saprophytic actinomycete population. Preliminary investigation showed, however, that only 10–30% of these types in soil are pigment producers. In practice it is therefore possible to eliminate almost 90% of saprophytic types by combining microscopic examination and tests for pigment reaction.

To isolate the scab organism from the more numerous saprophytic actinomycetes and bacteria on tubers, or in soil, a medium was sought that would combine the intense pigment production of Hollis' (4) medium with the bacterial suppression of low nutrient media such as that of KenKnight and Muncie (5). The formula finally selected was:

Tyrosine-casein-nitrate medium (TCN)	
Sodium caseinate	25 g
Sodium nitrate	10 g
L-tyrosine	1.0 g
Agar (Bacto)	15 g
Tap water	1000 ml

First dissolve sodium caseinate with gentle heating in 500 ml tap water, add other ingredients, and make up to 1 liter. Sterilize 20 minutes at 10 lb pressure. For plating, cool melted medium rapidly to 50°. pH about 6.8.

Other protein amendments, such as skim-milk powder, yeast extract, or peptones, were equal to sodium caseinate in stimulating pigment production, but they tended to encourage growth of spreading bacteria, especially proteolytic types that cleared the medium. Sodium caseinate produced a fine opaque medium in which the pigment from *S. scabies* stood out in contrast. Hollis (4) noted that diffusion of the pigment from colonies differed markedly with the protein supplement used. This effect, confirmed in our tests, seemed to be related to the relative absorption of pigment on protein particles. Sodium caseinate proved outstanding in absorbing the pigment close to the producing colony. With other protein materials, diffusion was sometimes so widespread that the colonies producing the pigment could not be recognized.

Sodium nitrate, used as an added N source, was satisfactory for actinomycete growth but did not favor bacteria. It was also found that clearing of the medium by bacteria, and even by *S. scabies*, was reduced by increasing the NO_3^- level. This proteolysis was completely prevented without reducing actinomycete counts by using 1.0% $NaNO_3$. No supplemental carbon source, such as sugar, was needed. The tap water used supplied enough inorganic salts. If distilled water is used, an inorganic salt combination such as used by Hollis (4) can be added.

In soil dilution plates, slightly higher counts of both bacteria and actinomycetes were obtained with this

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This investigation was supported in part by funds provided for biological and medical research by the State of Washington, Initiative Measure No. 171.

[2] Respectively, Microbiologist, Western Soil and Water Management Research Branch, Soil and Water Conservation Research Division, ARS, U. S. D. A.; and Senior Laboratory Technician, State College of Washington.

medium than with potato-dextrose or soil-extract agar. In spite of this, the melanin-producing actinomycetes were more easily counted because spreading bacteria were completely suppressed. TCN medium has been used routinely for successful isolation of *S. scabies* from tuber samples from many locations in the United States. It has also been used for plate counts of *S. scabies* in unsterilized soil artificially infested with the organism. For morphological examination of the isolates, they must be transferred to other media since both sporulation and growth of aerial mycelium is suppressed on the TCN medium.

Since there is evidence (4, 2, 9) that non-pigmenting strains of *S. scabies* sometimes occur, it should be noted that these will not be selected when TCN medium is used. These, however, appear to be rare. In our work, for example, every one of some 35 pathogenic isolates of *S. scabies* from different parts of the United States produce pigment in TCN agar.

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Prosser, Washington

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A ROOT-LESION-NEMATODE DISEASE OF CYMBIDIUM ORCHIDS[1]

S. A. Sher

SUMMARY

Pratylenchus scribneri Steiner is often found associated with a root and bulb rot and unthrifty top growth in *Cymbidium* spp. in nurseries in southern California. Infesting soil in which Cymbidiums were growing with 2 population levels of *P. scribneri* reproduced these disease symptoms. Similar plants treated with the water from which *P. scribneri* had been removed were similar in growth to untreated check plants.

A foliar nematode disease of orchids was reported in 1885 by Smith (4). In 1866 he further described this disease and illustrated the symptoms and the nematodes (5). Since then foliar nematodes (*Aphelenchoides* spp.) of orchids have often been reported in the literature (2). In 1895 de Man (1) described 3 species of nematodes from diseased pseudobulbs of orchids, but he did not consider them the cause of the disease. The first and only report of a root-lesion nematode (*Pratylenchus* sp.) from orchids is by Steiner (6), in 1927, when he found 1 female in the roots of a *Cattleya* sp. In 1957 a nematodes survey of orchids in Hawaii revealed a *Helicotylenchus* sp. and a *Paratylenchus* sp. associated with the roots of orchid plants (3).

A *Cymbidium* hybrid plant received in 1956 contained numerous specimens of the root-lesion nematode *Pratylenchus scribneri* Steiner in the soil, roots, and pseudobulb. Later examination of 17 potted plants from the same nursery in southern California revealed *P. scribneri* in 7 plants. *Cymbidium* plants in 8 additional nurseries in southern California were examined, and *P. scribneri* was found in plants from 5 of them. The nematodes were found in unthrifty plants exhibiting root and bulb rots. Soil around plants exhibiting excessive root damage was often negative for *P. scribneri*, but the nematodes could often be found in the roots and bulbs in the healthy-appearing tissue adjacent to necrotic areas (Fig. 1). As many as 3000 root-lesion nematodes (all stages) were present per g of bulb tissue. Although this survey was concerned with examination of *Cymbidium* plants, 6 *Cattleya* spp. and 5 *Cypripedium* spp. plants were examined. *P. scribneri* was found in the soil and roots of 1 of the *Cypripedium* plants.

→

Fig. 1–3. Cymbidium plants. **1)** Longitudinal section of pseudobulb showing lesions associated with numerous *P. scribneri* in tissue. **2)** Root on left from infested pot, on right from pot that received washings from which *P. scribneri* had been removed. **3)** From left to right, uninfested control plant; plant that received washings from which *P. scribneri* had been removed; plant infested with 200 *P. scribneri*; plant infested with 1000 *P. scribneri*.

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Paper No. 1100, University of California Citrus Experiment Station, Riverside.

1
2
3

Other plant-parasitic nematodes encountered in this survey were a *Paratylenchus* sp., *Helicotylenchus nannus* Steiner, and a *Helicotylenchus* sp.

To ascertain if *Pratylenchus scribneri* was responsible for the disease symptoms observed on *Cymbidium* and to measure any plant damage, the following test was undertaken.

MATERIALS AND METHODS.—Forty *Cymbidium* hybrids var. Michael Day, about 3 years old, were planted in 8-in. clay pots in a 1:1 mixture of steam-sterilized sand and peat moss. Ten pots were each infested with 200 *P. scribneri*, 10 pots each with 1000 *P. scribneri*, and 10 pots each with 50 ml of the water from which the *P. scribneri* were removed by washing through a 325-mesh soil screen 5 times, and 10 pots each with 50 ml of distilled water. The nematodes were obtained from *Cymbidium* bulbs ground in a blender and placed on a modified Baermann funnel to extract the nematodes. These were poured around the base of the plants in 50 ml of water.

The plants were randomized on a greenhouse bench, and all received a complete-nutrient-solution fertilizer once a week.

One year after inoculation, observations were made on the roots and tops. Two 50-cc soil samples from each pot were put on a modified Baermann funnel for 3 days. Plants were washed to remove the soil, and plant weights were taken. Root samples were taken from each series and cultured for fungi. Nematodes were extracted from the roots by placing 10 g of roots on a modified Baermann funnel for 3 days, and bulbs were examined for nematodes by placing portions of a bulb in a watch glass and teasing the tissue apart under a dissecting microscope.

RESULTS AND OBSERVATIONS.—The growth of plants in both groups infested with the *P. scribneri* was noticeably reduced at the end of the test (Fig. 3). Roots of all the infested plants were necrotic or almost completely absent (Fig. 3). There appeared to be more root destruction and less growth in the series containing 1000 *P. scribneri* per pot. Roots that were not completely destroyed had numerous necrotic lesions (Fig. 2), in which *P. scribneri* in all stages were found. *P. scribneri* were recovered from the soil in 9 of the 10 pots in the series infested with 200 nematodes per pot, and in 5 of the 10 pots in the series infested with 1000 nematodes per pot. No nematodes were recovered from the uninfested series (Table 1). Root and bulb samples were positive for *P. scribneri* in all the replications of the 2 infested series. The wet weights of both nematode-infested series of plants were significantly reduced at the 0.1% level below that of the 2 series without nematodes (Table 1).

A *Pythium* sp. was recovered from all the root samples, and was predominant in the series that received the washings and in the uninfested series. Other fungi recovered were *Fusarium moniliforme*, a *Pyrenochaeta* sp., a *Trichoderma* sp., and a *Cylindrocarpon* sp.[2]

TABLE 1.—*Infestation of Cymbidium with P. scribneri*

Treatment	Wet weight (g)	*P. scribneri* per 100 cc soil	*P. scribneri* per 10 g roots
200 *P. scribneri*	728.8**	6.6	73.0
1000 *P. scribneri*	563.4**	0.7	58.1
Washings	1272.6	0	0
Untreated	1200.6	0	0

**Significant at 0.1% level.

Only 2 plants flowered during this test. Both, in the untreated series, produced flower spikes of 8–10 flowers each.

The plants infested with *P. scribneri* not only were smaller and had necrotic roots and bulbs, but also had a number of yellow leaves—outer, older leaves, which turned yellow and died. Both infested series had at least 1 yellow or dead leaf per plant. This condition was not present in the untreated series or the series treated with the water from which *P. scribneri* had been removed.

The absence of *P. scribneri* in some of the soil samples, and its presence in the roots and bulbs of the plant, confirms earlier observations made in a nursery survey, where soil samples were negative and roots and bulbs positive. The number of root-lesion nematodes in the soil appears to be reduced when the roots and bulbs are in an advanced necrotic state.

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[2]Isolated by L. Davis, Department of Plant Pathology, University of California, Los Angeles.

CONTROL OF PRE-EMERGENCE DAMPING-OFF AND TWO LEAF-SPOT DISEASES OF SESAME BY SEED TREATMENT[1]

C. A. Thomas

SUMMARY

Orthocide 75 was the most effective of several seed protectants tested for protecting seedlings against pre-emergence damping-off by soil fungi. Control of *Pseudomonas sesami* on seed by treatment with streptomycin and of *Alternaria* sp. by treatment with other materials resulted in a significant reduction of leaf-spot diseases they cause in the field.

The development and commercial production of sesame (*Sesamum indicum* L.) as an oilseed crop are relatively new in the United States. Poor stands resulting from pre-emergence damping-off by soil fungi, and leaf-spots caused by *Pseudomonas sesami* Malkoff and *Alternaria* sp., are production problems particularly in the southeastern states.

The value and use of chemical fungicides for treatment of vegetable and cereal seeds are widely known. However, little or no information is available on the effectiveness and value of treatment of sesame seed, although sesame is one of man's oldest oilseed crops. This paper reports the results of experiments conducted at Beltsville, Maryland, in the greenhouse and in the field from 1954 through 1958. The tests were designed to determine the efficacies of chemical treatments for controlling pre-emergence damping-off and the 2 leaf-spotting pathogens.

MATERIALS AND METHODS.—Seed of the variety Kansas 10 produced at Beltsville, Maryland, was used in most tests. This seed was considered to be representative of that produced by dehiscent varieties grown under humid conditions, where natural infections of Alternaria and bacterial leaf spots occur. Seeds of Dulce and the indehiscent variety Delco, produced relatively free of the 2 pathogens at College Station, Texas,[2] were used in some tests. Viability of the seed in each lot was determined from germination tests of 100 seeds on moist filter paper in each of 4 Petri dishes held at 30°C for 7 days.

The seed treatment materials studied were Orthocide 75 (75% captan, *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide), Arasan (50% thiram, bis(dimethylthiocarbamoyl)disulfide), Arasan 75 (75% thiram), Spergon (96% chloranil, tetrachloro-*p*-benzoquinone), Dow 9B (32% zinc 2,4,5-trichlorophenoxide), Ceresan M (7.7% *N*-(ethylmercuri)-*p*-toluenesulfonanilide), Ceresan 75 (2.8% ethylmercuri 2,3-dihydroxypropyl mercaptide and 0.6% ethylmercuri acetate), Panogen 15 (2.2% cyano(methylmercuri)-guanidine), and Phygon (50% dichlone, 2,3-dichloro-1,4-napthoquinone). Two antibiotics, streptomycin and rimocidin, were used in studies on control of seed-borne pathogens.

Seeds in lots of 500 cc were treated with the materials listed. Ceresan M was applied as a slurry; Ceresan 75 and Panogen 15 were applied as liquids. These three treatments were stored in sealed flasks for 24 hours and then in paper bags until planted. The other seed protectants were applied as dusts and the treated seeds were stored in flasks. Most of the materials were tested at dosages of ¼ to 2 oz./bu. of seed. A wider range of dosages was used in some preliminary experiments, and dosages were changed as the work progressed. Certain chemicals were tested at maximum holding capacity of the seed to determine phytotoxicity. Seeds were soaked for 1 hour in solutions containing 250, 500, and 1000 ppm of streptomycin and 1000 ppm of rimocidin and then dried at room temperature.

Preliminary greenhouse tests, prior to 1956, consisted of plantings in flats, bench-beds, and 1-gal. glazed crocks containing naturally infested field soils, compost, or compost mixed with an equal volume of vermiculite. Three to 6 replications of 25–1000 seeds each were planted when the soils previously watered to field capacity had dried sufficiently so that the surface could be worked to a depth of ½ in. Seeds were planted in furrows ½ in. deep and then covered with loose soil that was firmed by hand. Effect of temperature was determined by placing the glazed crocks immediately after planting in constant-temperature chambers at 30°, 25°, 20°, or 15°C for 7 days and then at 30°C for 14 days.

After the preliminary trials, prior to 1956, greenhouse plantings were made in bench-beds containing compost mixed with an equal volume of vermiculite. Watering and planting were the same as in the preliminary tests. Most tests included 6 replications of 700 seeds each for each treatment. Tests were run when the soil temperature could be maintained at 20–25°C. All emerged seedlings were counted 10 days after planting.

Field trials of seed protectants for stand improvement were made each year, and after 1956 consisted of at least 4 replications of 700 seeds each. Tests were run usually in June when the soil temperature at the seed level averaged 20–25°C. About ½ inch of water was applied immediately after planting, by means of sprinkler irrigation. All emerged seedlings were counted 30 days after planting.

Isolations were made from damped-off seedlings in the greenhouse bench-beds and in the field plots to determine the causal organisms involved. Pathogenicity of fungi isolated most frequently was determined by the ability of individual isolates to cause pre-emer-

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[2] The author thanks Dr. M. L. Kinman for supplying the seed.

TABLE 1.—*Average percentage stands obtained in 1955–57 from seed of the sesame variety Kansas 10 treated with various protectants for control of pre-emergence damping-off caused mainly by Pythium ultimum in a greenhouse bench-bed of soil*

Seed protectant	Dosage oz./bu.	Mean percentage stand[a]
None		0.8
Orthocide 75	¼	60.2
	½	68.5
	1	73.0
Spergon	½	15.5
	1	31.7
	2	51.7
Arasan	½	42.9
	1	56.2
	2	63.2
Ceresan M	1	36.9
	2	48.5
Ceresan 75	1	34.4
	2	44.3
Panogen 15	1	41.0
	2	41.7
LSD (0.05)		8.2
(0.01)		10.9

[a] Average of 3 tests each consisting of 6 replications of 700 seeds. Seed in each test was more than 94% viable.

gence damping-off in artificially-infested steamed soil held at 20–25°C.

Studies were made of the efficacy of certain seed protectants and the two antibiotics for control of *Pseudomonas sesami* and *Alternaria* sp. on seed. Prior to 1956, observations on the development of the leaf spots on cotyledons and the first true leaves were made in the greenhouse and in the replicated field plantings. Subsequently disease occurrence was studied also in isolated plots planted entirely with untreated seed or seed given a single treatment. Treatments used were Orthocide 75, Arasan 75, and Spergon, each at ½ oz./bu., and the two antibiotics at dosages previously mentioned. Plantings were located at least 1 mile from other sesame and consisted of at least 10 rows 50 feet or more in length. Plantings were made each year at Beltsville, Maryland, and at Warsaw and Holland, Virginia, in 1956.

EXPERIMENTAL RESULTS.—Isolations from damped-off seedlings in greenhouse and field plantings yielded mostly species of *Pythium*, *Alternaria*, *Fusarium*, and *Rhizoctonia*. *Pythium ultimum* Trow was isolated more frequently than all other fungi combined and the pathogenicity tests indicated it was the most aggressive or virulent of the isolates tested.

Preliminary greenhouse experiments.—Results from plantings in composted soil mixed with vermiculite in bench-beds and glazed crocks were the most consistent. Poor moisture distribution and retention appeared responsible for lack of uniform results in other soils and in flats. Three to 6 replications of 700 seeds each in tests in bench-beds showed significant differences among most seed protectants.

Pre-emergence damping-off was greatest at 20°C. Stands obtained with untreated Maryland seed in 4 tests consisting of 4 replications of 50 seeds each were 69.9% at 30°C; 30.4% at 25°; 8.2% at 20°; and 48.3% at 15°.

Seed treated with Dow 9B produced poor and erratic stands in all preliminary tests. Phygon caused marked stunting of seedlings at dosages over 1 oz./bu. These seed protectants were not used in subsequent tests.

Greenhouse experiments after 1955.—Comparisons of the seed protectants at dosages of ½, 1, and 2 oz./bu. indicated that all materials caused significant increases in stand and that Orthocide 75 was the most beneficial, particularly at the lower dosage levels. Minimum effective test dosages of ¼ oz./bu. for Orthocide 75; ½ oz./bu. for Arasan 75 and Spergon; and 1 oz./bu. for the mercury compounds were established from results of repeated and separate experiments with each material at dosages of $\frac{1}{16}$, ⅛, ¼, ½, and 1 oz./bu.

The results of tests performed in 1955, 1956, and 1957 (Table 1) in which the protectants were compared at several dosage levels showed Orthocide 75 to be significantly more effective at a given dosage than the other materials. The only injurious effects noted were stunting of the seedlings and cupping of the cotyledons caused by Arasan 75 at 2 oz./bu.

Stands of Delco, an indehiscent variety, were improved less by seed treatment with Orthocide 75 than were stands of the dehiscent variety Dulce (Table 2). Slower germination of Delco seed and lower viability, which results from mechanical damage to the seed in threshing, presumably were responsible for the difference between the two varieties.

Field experiments.—Results of tests made prior to 1956 varied markedly from year to year. Seed protectants were effective in causing significant increases in stand only in the years when cool, wet weather occurred immediately after planting. Results were more consistent in the following years, when plantings were made only during cool periods and were irrigated. Stands obtained in 1958 (Table 3) were typical and showed Orthocide 75 to be superior to Arasan 75 and Spergon at the lower dosage levels. Arasan 75 was an effective seed protectant but appeared moderately phytotoxic at dosages greater than 1 oz./bu. Stands in field tests were never as good as those in greenhouse tests.

Control of seed-borne pathogens.—Plants of the

TABLE 2.—*Stand of 2 sesame varieties treated with Orthocide 75 for control of pre-emergence damping-off in a greenhouse bench-bed of soil*

Dosage oz./bu.	Mean percentage and stand[a]	
	Delco	Dulce
None	0.0	0.2
⅛	2.2	44.5
¼	5.5	53.8
½	10.5	65.6
1	18.4	69.0
2	28.3	75.3

[a] Average of 6 replicates of 700 seeds each. Delco and Dulce 43 and 84% viable, respectively.

variety Kansas 10 grown in the field at Beltsville for seed became affected with Alternaria and bacterial leaf spots before harvest. Plants grown in the field or greenhouse from such seed developed Alternaria spots on the cotyledons. Counts of these infections in the replicated greenhouse and field seed treatment tests indicated that all the protectants except Spergon gave good but not perfect control at the dosages tested. Rimocidin gave nearly perfect control when used at 1000 ppm as a seed soak.

Bacterial leaf spot did not develop sufficiently on cotyledons or first true leaves to permit evaluation of control in the replicated plantings.

Alternaria and bacterial leaf spots always developed and usually were severe by time of flowering in isolated plots planted with untreated seed. Bacterial leaf spot was never observed in isolated plots planted with the same seed that had been treated with streptomycin at 250, 500, or 1000 ppm. This was true in tests over a 3-year period in which at least 1 isolated plot each year was used for each treatment. None of the other seed protectants or rimocidin gave control. The development of Alternaria leaf spot in the isolated plots indicated that Orthocide 75, Arasan 75, and rimocidin gave good control in the seedling stage and often delayed severe infection until after flowering. Streptomycin and Spergon were not effective.

Table 3.—*Efficacies of 3 seed protectants for improving stand of the sesame variety Kansas 10 by control of pre-emergence damping-off in a field plot in 1958*

Material	Dosage oz./bu.	Mean percentage stand[a]
None		4.8
Orthocide 75	¼	31.4
	½	32.8
	1	33.8
	2	34.8
	max.	33.6
Arasan 75	¼	13.8
	½	24.1
	1	32.1
	2	33.7
	max.	38.7
Spergon	¼	10.8
	½	17.5
	1	26.1
	2	31.7
	max.	32.2
LSD (0.05)		2.1
(0.01)		2.8

[a] Average of 4 replications of 700 seeds each. Seed 96% viable.

Discussion.—Seed treatment was shown to be of considerable value for improving stands of sesame. There is ample evidence, however, that seed treatment may be of little value to sesame under conditions less adverse than those used here and that treatment may be ineffective under more severe conditions. Spergon and Arasan, which were widely used as seed protectants for sesame when the crop was first grown commercially in the United States, were found to be inferior to Orthocide.

Hoppe,[3] working with corn, and Hagedorn,[4] with peas, have shown that effectiveness of seed protectants in the field can be evaluated in the laboratory. Greenhouse tests with sesame appeared to indicate the relative value of seed protectants in the field.

Previous studies of sesame have demonstrated field control of leaf spots by seed disinfection. Nusbaum[5] was successful in controlling *Cercospora sesami* Zimm. with hot water. Brown and Streets[6] reported control of bacterial leaf spot by mercuric chloride seed treatment. Bacterial leaf spot in this study was controlled in isolated plots by treatment of infested seed with streptomycin. Loss from Alternaria leaf spot was reduced appreciably by seed treatment. Control of these diseases by use of clean seed and crop rotation was indicated. This was demonstrated on a large scale in 1958 on the Horseshoe Plantation at Tallahassee, Florida. Plantings made with infested seed produced on the plantation in 1957 were a complete loss from Alternaria leaf spot. Plantings made on new land with seed produced in Texas were free of leaf spot until plants approached maturity.

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SPREAD AND LIFE CYCLE OF THE LETTUCE POWDERY MILDEW FUNGUS[1]

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SUMMARY

Spread of the lettuce strain of powdery mildew, *Erysiphe cichoracearum*, in the Salinas Valley of California was primarily with the prevailing wind. The range of conidial dissemination appears to be at least 120 miles. Most spores were disseminated between noon and 4 P.M. The majority of conidia were disseminated in groups of 2 spores or more. The powdery mildew fungus overwintered in the Salinas Valley in the conidial stage, but surveys indicated that initial infections were induced by ascospores. Initial infections were observed only where perithecia had formed during the previous season. Perithecia burst upon absorption of water at 15–22°C, and ascospores from mature perithecia were viable and infected both lettuce (*Lactuca sativa*) and wild lettuce (*L. serriola*). Ascospores germinated both inside and outside the ascus. Development of the mildew colony was illustrated.

In the United States, powdery mildew of cultivated lettuce (*Lactuca sativa* L.), incited by *Erysiphe cichoracearum* DC. ex Mérat, has occurred regularly each year since 1951 (4, 7) in an area centered in the Salinas Valley of California (6). The disease in this country probably originated in the Salinas Valley (6), but now occurs in other lettuce-growing areas in California and Arizona (6). Symptoms and damage caused by this disease are described and illustrated elsewhere (2, 3, 5, 6, 7).

An investigation was conducted to study the establishment and development of the pathogen in the field and to determine its life cycle in the California environment.

OBSERVATIONS AND EXPERIMENTAL RESULTS.—*Areas of initial infection and spread of the disease.*—Areas of initial infection, and the direction and distance of spread of the powdery mildew fungus, were studied in the Salinas Valley by means of monthly surveys. The manner of determining the percentage and severity of infection has been previously described (6). In surveys prior to 1955, perithecia were found consistently, and most abundantly, near Salinas, but were also found in scattered locations from virtually one end of the Salinas Valley to the other (Fig. 1). During the winter of 1954-1955, over-wintering lettuce fields were carefully examined for active powdery mildew colonies. Such colonies were found in only a few locations during the winter months (Fig. 1) and the plants in these fields were turned under early in the spring (February 28, 1955) to make way for new plantings. At this time, however, young lettuce was quite plentiful in the valley. Such fields adjacent to old fields with active mildew were observed carefully and repeatedly from February 28 to June 25, but no mildew was found. On July 21, powdery mildew was observed on lettuce at 2 locations, which were 5 and 8 miles southeast of the field where conidia had been observed in February. Perithecia had been found at these 2 locations for several years. A study was initiated to determine the direction, distance, and rapidity of spread from one of these areas. In one of these fields perithecia had been quite numerous, and the percentage of infection of lettuce plants increased from 0 to 85% in 26 days (June 25 to July 21). During this same period the fungus spread to adjacent fields, primarily with the prevailing northwesterly wind, infecting 20% of the plants in some fields. By August 25, 100% of the plants were infected in the field where perithecia had been the most numerous. Eighty per cent of the lettuce plants in the region between the 2 locations where the first infections occurred (about 3 miles apart) were infected by September 25.

At 2 additional locations, 15 and 30 miles southeast of the 2 lettuce fields where lettuce plants were first infected, 100% and 82% of the plants were infected by September 25. Perithecia had formed previously in both locations. Plants in the area between these 2 locations were free of mildew in September. Movement of the disease to the northwest (toward the Pacific Ocean), from the northwesternmost field where initial infections occurred, was detectable in September. From September 25 to November 9 the distribution of mildew did not change appreciably, though it increased in severity. In fields lacking infected plants on Sep-

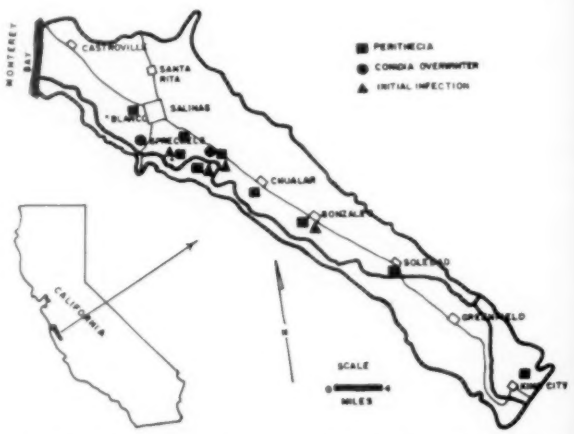

Fig. 1. Lettuce-growing area of the Salinas Valley, with regions of perithecial formation, conidial overwintering, and initial infections indicated for lettuce powdery mildew (1954–55). The initial infections at Gonzales are infections of wild lettuce by the lettuce strain rather than infections of cultivated lettuce.

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TABLE 1.—*Number of conidia of lettuce powdery mildew trapped on glass slides in the Salinas Valley at various times of the day*

Time and date	Total spores	Total groups of spores	Total spores in groups of 2 or more	Total single spores	% germ.
8:00 PM 8/24/55 to 8:00 AM 8/25/55	1[a,b]				
8:00 AM 8/25/55 to 3:00 PM 8/25/55	22[a,b]				
3:00 PM 8/25/55 to 8:00 PM 8/25/55	18[a,b]				
7:40 PM 10/17/55 to 8:15 AM 10/18/55	36[a,c]	6	27	9	42
8:15 AM 10/18/55 to 4:30 PM 10/18/55	229[a,c]	36	187	42	34
4:30 PM 10/18/55 to 8:45 PM 10/18/55	5[a,c]	1	3	2	0
8:45 PM 10/18/55 to 9:00 AM 10/19/55	23[a,c]	3	10	13	79
9:00 AM 10/19/55 to 12:30 PM 10/19/55	41[a,c]	10	31	10	54
12:30 PM 10/19/55 to 4:20 PM 10/19/55	204[a,c]	42	142	62	45
5:00 PM 11/ 8/55 to 2:15 PM 11/10/55	323[d]		201	122	27

[a]Spores trapped on glass slides placed horizontally on wooden blocks.
[b]The average number of colonies of powdery mildew per basal leaf of the lettuce plants was 11.
[c]The average number of colonies of powdery mildew per basal leaf of the lettuce plants was 67.
[d]Spores trapped on glass slides placed vertically on wooden stakes.

tember 25, 50-100% were infected by November 9. Further movement of powdery mildew to the northwest was detected, but infection was severe only in fields where the incidence of lettuce mosaic was high.

In general the disease was most severe in the areas where perithecia had been observed previously.

Dissemination of conidia.—During the 1955 growing season, studies were conducted in several fields of differing disease severity to determine the amount and pattern of conidial dispersal. Conidia were collected on dry microscope slides, to which they readily adhere. Slides were placed horizontally on 1-in.-thick wooden blocks resting on the ground in the center of lettuce beds, or were mounted vertically on 3-ft.-high wooden stakes and directly facing the prevailing wind. The sampling blocks and stakes were distributed in a straight line, but at an angle to the lettuce beds of about 45°. Samples were collected well in from the edge of the lettuce fields and at 10-bed intervals. Each sample (3 slides) was collected at various times throughout the day and night. The slides were placed on glass rods in Petri dishes containing 5 cc of distilled water so that RH (relative humidity) would be maintained near 100%, preventing conidia from drying out and permitting them to germinate. All slides were examined 2 days later for determination of the number of spores trapped and the percentage of germination.

The greatest numbers of conidia were trapped between about 12:00 NOON and 4:00 P.M. (Table 1). A few spores were trapped at night, but much less than during the day. Seventy per cent of the conidia trapped occurred in groups of 2 or more, with as many as 22 spores in a group. Fig. 2 shows a typical example of dissemination of several spores as a single large group. The high percentage of spores in groups could possibly have been the result of the horizontal position of the slides, which might have favored the collection of heavier spore masses. However, a similar proportion was found on the vertical slides.

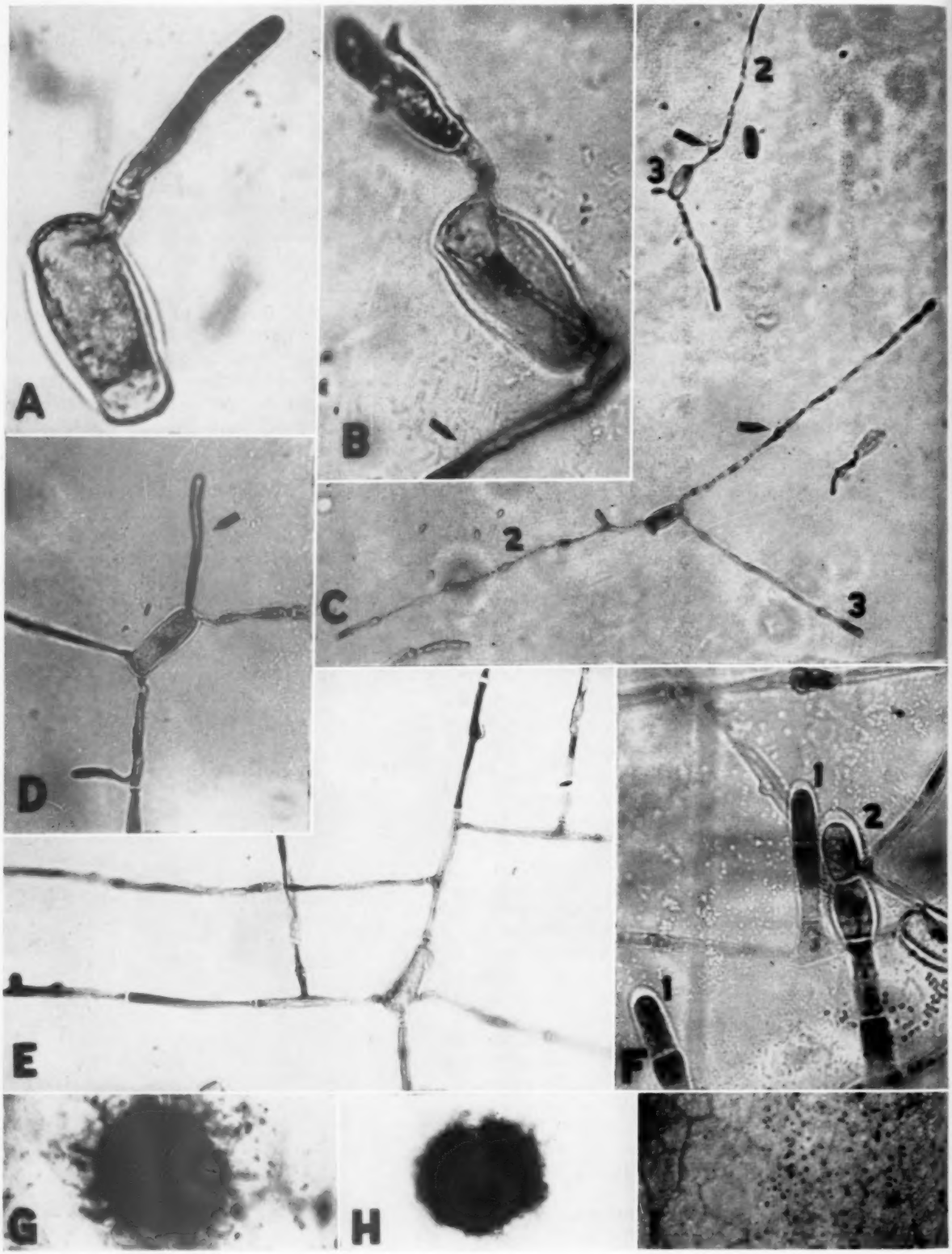
It was estimated that at least 2×10^8 conidia were disseminated over an acre of lettuce every 24 hours during the period when mildew colonies on the basal leaves averaged 67 per leaf.

Development of the mildew colony.—Development of the colony was studied in detached leaf culture. Healthy lettuce leaves of the Great Lakes variety were floated on 10 cc of a 3% sucrose solution in Petri dishes, inoculated with freshly produced conidia with a camel's-hair brush, and incubated at 22°C. A continuous source of illumination of 300 ft-c was provided at the leaf surface. Celloidin strippings (1) were taken after various intervals, placed for 30 minutes in .05% cotton blue in lactophenol, and then mounted

Fig. 2. Conidia of lettuce powdery mildew trapped as a group in the Salinas Valley. Picture was taken of spores as they occurred on sampling slide from the field.

in clear lactophenol for observation.

The germ tube (Fig. 3-A) occasionally developed a prominent appressorium (Fig. 3-B), but more commonly tended to enlarge only slightly (Fig. 3-A). A septum was formed, and the host was directly penetrated by an infection peg averaging 1.5 μ in diameter.



Infection was indicated when the primary infection hypha (Fig. 3-B) formed, because it usually did not form where infection was impossible, i.e., glass slides, water agar, etc. Haustoria were globose and occasionally branched. Two, and sometimes 3, haustoria formed in a single cell when mildew development was profuse. The secondary infection hypha, arising from the primary appressorium, and the primary infection hypha elongated and formed distinct appressorial enlargements, intermittently distributed along each hypha (Fig. 3-C). After this elongation, a tertiary hypha formed (Fig. 3-C), followed by branching of the secondary hypha. A quaternary hypha generally developed (Fig. 3-D), followed by branching of all hyphae (Fig 3-E). A single conidium usually formed 4 germ tubes, but sometimes 3 or 5. Conidiophore initials appeared and gradually enlarged to about twice the width of the mycelium (Fig. 3-F). If undisturbed, conidia were formed in chains of as many as 20 spores.

Function of perithecia and ascospores.—Tests were made in the laboratory to determine whether perithecia were functional and whether ascospores were viable and could infect lettuce and wild lettuce. During the winter of 1955–56, perithecia of the wild-lettuce strain of *E. cichoracearum* were collected from dead leaves of wild-lettuce plants. In addition, perithecia of the lettuce strain were obtained from pressed *L. sativa* and *Silybum marianum* Gaertn. leaves (*S. marianum* infected in the laboratory produced perithecia in some instances) that had died before being pressed. The wild-lettuce leaves were stored 3 months at 10°C and about 60% RH. The lettuce and *S. marianum* leaves had been stored 18 months at 22°C and about 50% RH. Leaf tissue with perithecia was examined to make certain that none of the perithecia had burst, placed on moistened filter paper in Petri dishes, and held at 22°C.

Perithecia of both strains began breaking within 24 hours (Fig. 3-G,H) and continued to break for several days. In similar tests the highest percentage of breakage was 15 in any one test. It is probable that perithecial breakage was caused by hydrostatic pressure due to absorption of water. Maturity of perithecia of both the lettuce and wild-lettuce strains of mildew could be gauged by the number of shriveled perithecia on the leaf, maturity being inversely proportional to the number that shriveled. Perithecia from green leaves were usually not functional, and the ascospores contained within were not viable. In contrast, ascospores were viable when taken from perithecia on leaves that had been allowed to die on the plants. Ascospores formed short germ tubes on glass slides, and germinated inside the ascus as well. Ascospores that germinated in the ascus formed a penetration peg that penetrated the ascus wall.

Several attempts were made to determine whether ascospores of this fungus could infect a host resulting in the formation of mildew colonies.

Mature perithecia of the lettuce strain of mildew were produced in the laboratory (13°C, 60% RH, and 900 ft.-c of illumination at the tops of the plants) on stems of wild lettuce (*L. serriola* L.). The majority of perithecia produced under these conditions appeared to be mature. Small pieces of the dead stem (dead tissue was used to prevent possible contamination from conidia) containing perithecia were removed with a razor blade, placed on pieces of circular filter paper, and inserted into a Petri-dish lid. The filter paper was moistened with distilled water and inverted over detached lettuce or wild-lettuce leaves floating on distilled water in the lower half of the Petri dish. The dishes were placed in the laboratory at 22°C. Continuous illumination of 300 ft-c was provided. All leaves used in these tests were obtained from healthy plants grown in a greenhouse that was checked frequently for the presence of contaminating mildews (on wild lettuce only). No contamination was observed.

Another method of obtaining ascospore infections was also used. It was similar to the above method except that 50 perithecia were transferred from the dead stem pieces to circular filter papers, which were placed in a Petri dish cover inverted over detached wild-lettuce leaves and held at 15°C. This method cut down the amount of contamination from other fungi, which, with the other method, often overgrew the moistened stem tissue.

Infection of lettuce and wild-lettuce leaves resulted from both methods. Measurements of the spores from which the colonies arose indicated that an ascospore was involved in every case.

It is evident that ascospores are viable and functional and constitute a means by which the organism may survive the winter and bring about infections in the following season.

DISCUSSION AND CONCLUSIONS.—Since infections of lettuce powdery mildew appear first in the same areas each year, at sites where perithecia had abundantly formed during the previous season, it appears that perithecia are functional and that ascospores play a part in the disease cycle. This was substantiated by the fact that, although the fungus can overwinter in the asexual stage, initial infections during these studies were not due to conidia, for initial infections did not occur until 3 months after the overwintering plants had been destroyed. In addition, it was shown that

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Fig. 3. Perithecia and stages in the development of the lettuce powdery mildew colony from germination to sporulation. **A-F)** taken from celloidin strippings stained in cotton blue; **G-I)** from leaf tissue. **A)** Germ tube and primary appressorium, which is the area from the septum to the tip of the germ tube. **B)** Primary infection hypha (arrow); note the unusually large primary appressorium formed on the germ tube. **C)** Secondary hypha formed from the germ tube (2), formation of the tertiary hypha (3), and appressoria formed along hyphae (arrows). **D)** Quaternary hypha (arrow). **E)** Branching of hyphae. **F)** Primary (1) and secondary (2) conidia. **G** and **H)** Perithecia broken *in situ* by absorption of water at 22° C. **I)** Perithecia formed on lettuce in the field.

perithecia and ascospores are functional.

More conidia were dispersed when high wind and low RH occurred simultaneously in the afternoon (5). Wind velocity and RH conditions appear to explain the fact that greater numbers of conidia were trapped in the afternoon. These results are similar to those reported by Yarwood (8) with *Erysiphe polygoni* DC. Yarwood (8) stated that dissemination of conidia was presumably a passive process, dependent on the maturity of the conidia, on the dryness of leaf and fungus surfaces, and on wind. He suggested that liberation of conidia would be reduced at night, because of higher RH and less wind. The writer observed that chains of conidia and individual spores at high RH (100%) stuck together, preventing the detachment of chains or single spores, whereas chains of spores formed at lower humidities (50–60%) did not adhere to each other. RH therefore could affect to a large extent the number of spores set free from mildewed leaves.

Powdery mildew infections on lettuce at Gilroy, San Jose, Concord, and Brentwood, north of the Salinas Valley, appear almost simultaneously. This indicates that spore showers probably occur over a wide area and that the source of the spores is the Salinas Valley. This view is further supported by the fact that perithecia have not been found in lettuce areas north of the Salinas Valley. The effective range of conidial dissemination appears to be at least 120 miles, i.e., the distance from the Salinas Valley to Brentwood and Concord, in northern California.

Observations in the laboratory indicate that spores in clusters are more resistant to desiccation than are spores occurring singly. Dissemination of conidia in groups (chiefly single and paired conidia clustered together) could be a decided advantage to the fungus in that more viable conidia would reach the host than would reach it if conidia were disseminated singly. Dissemination of conidia solely as chains (3 or more conidia in a chain), on the other hand, does not appear to be of any advantage to the fungus. Observations in the laboratory indicate that only the end spore (oldest spore) in a conidial chain is capable of infecting the host, even though other conidia in the chain germinate.

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HOST-PATHOGEN INTERACTION OF THE GENES FOR RESISTANCE IN HORDEUM VULGARE AND FOR PATHOGENICITY IN ERYSIPHE GRAMINIS F. SP. HORDEI[1]

J. G. Moseman[2]

SUMMARY

The interaction of the genes conditioning resistance in the barley host and the powdery mildew pathogen was determined. For each of the genes Ml_g and Ml_k, which condition resistance in Goldfoil (C.I. 928)[3] and Kwan (C.I. 1016) barley varieties, respectively, there was a complementary gene for virulence in culture CAN12 of race 12 of *Erysiphe graminis* f. sp. *hordei*. The gene complementary to Ml_g was designated V_g, and the one complementary to Ml_k was designated V_k. By study of progeny of the cross of cultures CAN12 and 21.1 of race 9, avirulent on Goldfoil and Kwan, it was shown that the genes V_g and V_k were inherited independently.

INTRODUCTION.—As early as 1905 Biffen (1) established that the inheritance of resistance to disease-inciting organisms in plants followed Mendelian laws. Since then the relationship of the host and pathogen has been studied extensively. It has been found that host-pathogen relationships include directly or indirectly most of the fundamental problems of infectious diseases. Although there have been extensive studies on physiologic specialization, pathogenic variability, and inheritance of genes conditioning resistance, new varieties resistant to a certain fungus frequently become susceptible to new pathogenic strains of it a few years after their release. This suggests the need for a new approach to study of the problem.

Flor (4) proposed a complementary gene-for-gene relationship between rust reaction in the host *Linum usitatissimum* L. and pathogenicity in the flax rust fungus *Melampsora lini* (Pers.) Lev. This study was undertaken to determine whether the same relationship exists between the genes for resistance in the host *Hordeum vulgare* L. and for pathogenicity in the pathogen *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal. This study differs, however, in one important genetic feature, namely, in flax and flax rust the organisms in the host-pathogen relationship are diploid and dicaryon, whereas, for barley and barley powdery mildew, the organisms are diploid and haploid, respectively. The interaction was studied of the genes that condition resistance in the varieties Goldfoil (C. I. 928)[3] and Kwan (C. I. 1016) and of the genes for pathogenicity in cultures CAN12 of race 12 and 21.1 of race 9.

REVIEW OF LITERATURE.—The barley varieties Goldfoil and Kwan were each found to have one gene conditioning resistance to the culture of race 3 of powdery mildew present in California. In 1937, Briggs and Barry (2) designated the gene in Goldfoil that conditioned resistance to a culture of race 3 of powdery mildew, Ml_g. In 1938, Briggs and Stanford (3) identified a gene, which they designated Ml_k, in Kwan that conditioned resistance to this culture of race 3, and they showed that the genes Ml_g and Ml_k were inherited independently.

There have been numerous investigations on physiologic specialization and pathogenic variability in *Erysiphe graminis* f. sp. *hordei*, yet genes for pathogenicity have been identified in only a few studies. The 2 genes for pathogenicity in the present study were first reported (5) in 1957. At the same time Powers and Sando (10) reported isolating 2 genes in *E. graminis* DC. f. sp. *tritici* Em. Marchal for pathogenicity on wheat.

MATERIAL AND METHODS.—Seed of the varieties Goldfoil (C. I. 928) and Kwan (C. I. 1016), which are susceptible to culture CAN12 of race 12 and resistant to culture 21.1 of race 9, were obtained from C. W. Schaller, in California. These were from the supply used in studies in which the genes Ml_g and Ml_k were isolated. Seed of the variety Manchuria (C.I. 2330) was obtained from G. A. Wiebe.

The cultures CAN12 of race 12 and CR3 of race 3 are the cultures used in earlier studies (7, 8). Culture CAN12 originated from a single ascospore from cleistothecia on plants grown by W. J. Cherewick in the greenhouse at Winnipeg, Canada. Culture CR3 is a single conidial subculture of the culture of race 3 used to identify the genes Ml_g and Ml_k. Culture 21.1 of race 9 originated from a single ascospore from a plant grown in Pennsylvania.

Since it was found in the studies reported by Powers and Moseman (9) and additional unpublished tests that both *Erysiphe graminis* f. sp. *tritici* and *Erysiphe graminis* f. sp. *hordei* are heterothallic, it was possible to obtain crosses of the pathogen by the following method: One or 2 plants of the susceptible variety Manchuria were grown in 5- or 6-in. clay pots for 4–6 weeks in a section of the greenhouse free from powdery mildew. The powdery mildew was kept out of this section by placing sulphur on the steam pipes at weekly intervals. Before inoculations were made, a plastic cylinder 36 in. high, with the upper end covered with 4 layers of cheesecloth, was placed around the plants. Inoculations were made by removing the cheesecloth from the top of the cylinder, and spores of each culture to be crossed were allowed to fall on the plants. The inoculated plants were placed in a

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[3]C.I. refers to the accession number of the Cereal Crops Research Branch, Crops Research Division.

TABLE 1.—*Seedling reactions of F_3 families from crosses of barley varieties Goldfoil and Kwan with Manchuria to inoculation with cultures CR3 and 21.1 of Erysiphe graminis hordei*

Cross	Culture	Number of F_3 families [a] Resistant	Segregating	Susceptible	Ratio	Value of P for ratios indicated
Manchuria × Goldfoil	CR3	9	25	10	1:2:1	>0.50
	21.1	9	25	10	1:2:1	>0.50
Manchuria × Kwan	CR3	12	23	11	1:2:1	>0.95
	21.1	12	23	11	1:2:1	>0.95

[a] All the F_3 families were ioculated separately with cultures CR3 and 21.1, and found to react the same.

greenhouse kept at about 70°F. Cleistothecia formed in 4–6 weeks on plants inoculated with cultures that were of compatible sexual types.

Leaves with cleistothecia were harvested and stored at 10°C in envelopes in a dry refrigerator. The method for obtaining ascospores from cleistothecia was described in detail by Moseman and Powers (6). For this study the ascospores were allowed to fall on and infect seedlings growing in 3-in. clay pots surrounded by lamp chimneys. Sections of leaves with individual pustules were cut out and floated on a 2% sucrose solution for 48 hours and then transferred either to other plants growing in 3-in. clay pots surrounded by lamp chimneys or to plants growing in 300-mm test tubes.

Inoculations and tests of the reaction of the plants to infection were made in a growing room at 66–69°F, with 550 ft-c of light from cool, white fluorescent tubes for 16 hours a day. The same F_3 families from the crosses Manchuria × Goldfoil and Manchuria × Kwan were inoculated separately with cultures CR3 and 21.1 for comparing the reactions of the families to the 2 cultures. The number of plants tested per F_3 family was 15 for a susceptible family and 25 for a segregating or a resistant family.

RESULTS.—Seedling reactions of F_3 families from the crosses of Manchuria with Goldfoil and with Kwan to inoculation with cultures CR3 and 21.1 of *Erysiphe graminis* f. sp. *hordei* are shown in Table 1. Previous tests (2, 3) of progeny from crosses of Goldfoil and Kwan with Atlas showed that each variety has 1 gene conditioning resistance to culture CR3. Data in Table 1 indicate that each variety, when crossed with Manchuria, has 1 gene conditioning resistance to culture CR3. Since all F_3 families from the 2 crosses reacted the same to cultures CR3 and 21.1, it is assumed that the same gene conditions resistance to both cultures. Since the seed of Goldfoil and Kwan were from the same source used to identify genes Ml_g and Ml_k, and culture CR3 is a subculture of the culture from that study, it is assumed that the 2 genes conditioning resistance to cultures CR3 and 21.1 are Ml_g from Goldfoil and Ml_k from Kwan.

Genes for reaction to powdery mildew in the host are identified by the pathogenicity of specific cultures of the pathogen. Genes for pathogenicity in the pathogen are identified by the reaction of specific varieties of the host. Designations of the genes for resistance in barley varieties Goldfoil and Kwan, and the complementary genes for pathogenicity in powdery mildew cultures CAN12, CR3, and 21.1 are shown in Table 2. The genes in barley for reaction to powdery mildew are designated by Ml for a dominant gene and ml for the recessive allele. The gene locus is identified by a subscript letter following the letters Ml. These subscript letters are small, in conformity with the designation previously used of genes conditioning resistance to powdery mildew in barley. Alleles at a gene locus would be indicated by a superscript number following the letter identifying the gene locus. The genotype of Goldfoil is $Ml_gMl_gml_kml_k$ and for Kwan $ml_gml_gMl_kMl_k$. The genes in the pathogen for pathogenicity are designated by either letter A or V followed by the subscript for the complementary gene locus in the host. The letter A indicates that the gene is avirulent to the complementary gene for resistance in the host, and V indicates it is virulent. The subscript letter identifies the complementary gene locus in the host. The respective genotypes of cultures CAN12, CR3, and 21.1 are V_gV_k, A_gA_k, and A_gA_k.

Table 3 shows the frequency of cultures with various genotypes derived from the cross of cultures CAN12 and 21.1 as determined by reaction types resulting on hosts of known genotypes. There were 26 cultures virulent and 23 cultures avirulent on Goldfoil, which has the gene Ml_g for resistance, and 28 cultures virulent to 21 cultures avirulent on Kwan, which has the gene Ml_k for resistance. These frequencies of virulent to avirulent cultures fit the 1:1 ratio expected when there is 1 gene for pathogenicity. All the cultures were virulent in the variety Manchuria, which does not possess genes for resistance to either of the cultures in the cross. As determined by the reaction of the 3 varieties with known genotypes, there were 21 cultures with the genotype of the parent cultures and 28 cultures whose genotype differed from the parent cultures. The frequency of the 4 possible genotypes

TABLE 2.—*Designation of genes for resistance in barley varieties Goldfoil and Kwan and for pathogenicity in powdery mildew cultures CAN12, CR3, and 21.1*

Barley			Powdery mildew culture and genotype (N) [b]		
Variety	Genotype	(2N) [a]	CAN12	CR3	21.1
Goldfoil	Ml_gMl_g	ml_kml_k	V_g	A_g	A_g
Kwan	ml_gml_g	Ml_kMl_k	V_k	A_k	A_k

[a] The letters Ml indicate genes for reaction to powdery mildew. Capital letter Ml is for dominant genes and small letter ml is for their recessive alleles. The subscript letters identify the gene locus. Alleles at a gene locus would be indicated by superscript numbers following the letter identifying the gene locus.

[b] The letters A and V indicate the avirulence and virulence genes for pathogenicity, respectively. The subscript letters identify the complementary gene for resistance in the host.

TABLE 3.—*Frequency of cultures with various genotypes derived from the cross of cultures CAN12 and 21.1 as determined by reaction types on hosts with known genotypes*

Variety	Genotype of host	Reaction types[a] on genotype of pathogen[b]				Ratio R:S[c]		Value of P for ratios indicated
		V_gV_k	V_gA_k	A_gV_k	A_gA_k	Obs.	Exp.	
Goldfoil	Ml_gMl_g ml_kml_k	S	S	R	R	23:26	1:1	>0.60
Kwan	ml_gml_g Ml_kMl_k	S	R	S	R	21:28	1:1	>0.30
Manchuria	ml_gml_g ml_kml_k	S	S	S	S	0:49	0:1	>0.99
Total each genotype		13	13	15	8		1:1:1:1	0.50

[a] Reaction type S = susceptible; R = resistant.
[b] Genotype V_gV_k is for culture CAN12 and A_gA_k is for culture 21.1.
[c] Ratio of resistant and susceptible reaction types expected if the hypothesis is valid that there is a complementary gene for pathogenicity for each gene that conditions resistance.

fits the 1:1:1:1 ratio expected if the 2 genes for pathogenicity are inherited independently.

To verify that the culture CAN12 has 1 virulent gene V_g complementary to the gene Ml_g in the variety Goldfoil, and another virulent gene V_k complementary to the gene Ml_k in the variety Kwan, cultures of the 4 genotypes were crossed with the parent cultures and with each other. Genotypes of the cultures resulting from these crosses as determined by the reaction of varieties of known genotypes are shown in Table 4. At least 8 progeny were tested from all but 1 of the 19 crosses. For each of the crosses the genotypes of the cultures obtained were those expected. A total of 231 progeny were tested.

DISCUSSION.—Whether an organism is homothallic or heterothallic determines how it can be used in genetic studies. Since *Erysiphe graminis* f. sp. *hordei* is heterothallic, sexual combination will occur only when cultures of opposite sexual compatibility groups are combined. Therefore, a culture derived from a single spore cannot be selfed, and mixtures in a culture, if they are of opposite compatibility groups, can be detected by placing the culture on plants under conditions in which cleistothecia are formed. If the cultures involved in a cross are checked for mixtures of sexually compatible cultures, then cleistothecia formed are the result of the combination of the 2 cultures. Two cultures with the same sexual compatibility group will not cross. This often prevents the investigator from obtaining desired crosses between certain cultures.

A determination of the pathogenicity of progeny from a cross between 2 cultures that differ by 2 genes for pathogenicity shows the relationship of those genes. The phenotypic expression of the reaction on the host also identifies its genotype, since the pathogen is haploid. Therefore it is not necessary, as with diploid or dicaryon species, to progeny-test the organism to determine its genotype. The linkage relationship of the genes can be determined by comparing the number of progeny from a cross that are similar to the parents with the number that are dissimilar. This could be important in predicting the frequency of occurrence in nature of specific pathogenic types.

The complementary relationship of the specific genes in the host with specific genes in the pathogen could be

TABLE 4.—*Frequency of cultures with various genotypes derived from crosses of cultures of 4 genotypes with parent cultures CAN12 and 21.1 and with each other as determined by reaction types on hosts with known genotypes*

Cross of cultures[a]	Genotype		Number progeny with genotype				Expected ratio of genotype			
	First parent	Second parent	V_gV_k	V_gA_k	A_gV_k	A_gA_k	V_gV_k	V_gA_k	A_gV_k	A_gA_k
CAN12 × 21.1	V_gV_k	A_gA_k	13	13	15	8	1	1	1	1
CAN12 × 37	V_gV_k	A_gA_k	1	5	3	1	1	1	1	1
CAN12 × 3	V_gV_k	V_gA_k	9	4	0	0	1	1	0	0
CAN12 × 20	V_gV_k	A_gV_k	6	0	6	0	1	0	1	0
CAN12 × 7	V_gV_k	V_gV_k	9	0	0	0	1	0	0	0
21.1 × 35	A_gA_k	A_gA_k	0	0	0	10	0	0	0	1
21.1 × 23	A_gA_k	V_gA_k	0	10	0	4	0	1	0	1
21.1 × 19	A_gA_k	A_gV_k	0	0	5	4	0	0	1	1
21.1 × 40	A_gA_k	V_gV_k	1	2	2	3	1	1	1	1
37 × 35	A_gA_k	A_gA_k	0	0	0	11	0	0	0	1
37 × 23	A_gA_k	V_gA_k	0	6	0	3	0	1	0	1
37 × 19	A_gA_k	A_gV_k	0	0	1	3	0	0	1	1
37 × 40	A_gA_k	V_gV_k	2	2	4	2	1	1	1	1
3 × 23	V_gA_k	V_gA_k	0	10	0	0	0	1	0	0
3 × 40	V_gA_k	V_gV_k	6	3	0	0	1	1	0	0
23 × 20	V_gA_k	A_gV_k	3	5	2	2	1	1	1	1
19 × 20	A_gV_k	A_gV_k	0	0	10	0	0	0	1	0
20 × 40	A_gV_k	V_gV_k	7	0	5	0	1	0	1	0
7 × 40	V_gV_k	V_gV_k	10	0	0	0	1	0	0	0

[a] Cultures with the identical pathogenicity are CAN12, 7 and 40; 21.1, 35 and 37; 3 and 23; and 19 and 20.

very useful in genetic studies. It has often been assumed that varieties that react the same to several pathogenic strains of the fungus possess the identical genes for resistance. It has likewise been assumed that pathogenic strains of the fungus that have the same pathogenicity on several varieties possess the same genes for pathogenicity. If the genes in one organism are known, the relationship of genes in the other organism can be determined.

If the complementary gene-for-gene relationship is valid, then the number and relationship of genes conditioning resistance to powdery mildew in barley varieties can be determined by the pathogenic reaction of progeny from a cross between 2 cultures of *Erysiphe graminis* f. sp. *hordei* that differ in their pathogenicity on those varieties. The number of genes controlling pathogenicity in the cultures can be determined by the pathogenicity of progeny from the cross, and the same number of genes would likewise be conditioning resistance in the host. If all cultures from this cross reacted similarly on 2 varieties, then the pathogenicity of those 2 cultures on those varieties is controlled by the same gene, and the varieties would have the same gene conditioning resistance to those 2 cultures. There is the possibility that 2 genes may be closely linked and segregate as a single gene, as may happen in studies of inheritance of genes conditioning resistance in the host. However, since the pathogen is a haploid organism, closely linked genes could be detected more readily than in diploid or dicaryon organisms.

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POSTHARVEST DECAY OF PEACHES AS AFFECTED BY TEMPERATURES AFTER HYDROCOOLING IN WATER OR DOWICIDE A SOLUTIONS[1]

T. T. McClure and W. L. Smith, Jr.[2]

SUMMARY

Peaches inoculated with *Monilinia fructicola* (the brown rot fungus) or *Rhizopus stolonifer* (the Rhizopus rot fungus) were hydrocooled in water or in a 0.1% Dowicide A solution, held 3 days at 40°, 45°, 50°, 55°, or 60°F, and placed at 75° for 3 or 6 days. The percentages of brown rot and Rhizopus rot after 3 days at 75° were less in peaches, previously held at 40° than in peaches held at 60°. After 6 days at 75° Rhizopus rot was still somewhat less in peaches previously held at 40° than in peaches previously held at higher temperatures. The percentage of brown rot at this time was about the same, regardless of previous holding temperatures. Dowicide A reduced both rots, but was much less effective when the holding temperature was 60° than when it was 55° or lower.

INTRODUCTION.—Hydrocooling peaches has become a common practice in the southeastern section of the United States. The treatment reduces the temperature of the fruit rapidly, and is believed to delay ripening and decay during holding or transit. In commercial practice, however, peaches are usually allowed to warm to room temperature during retail marketing. Information is limited on the development of decay of peaches at warm temperatures after removal from cold temperatures. As reported earlier (3), peaches hydrocooled and held 5 days at 40°F developed less Rhizopus rot at 75° than comparable peaches not held at the low temperature. Decay was still less when peaches were hydrocooled in a fungicide solution before holding at 40°. The present study expands the

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previous work and reports the effect of various temperatures that may occur during holding or transit on the amounts of brown and Rhizopus rots that develop on hydrocooled peaches after they are warmed to room temperature.

MATERIALS AND METHODS.—Peaches obtained from wholesale houses in Washington, D. C., were sorted for approximate uniformity of ripeness and freedom from decay or other damage. They were composited into experimental samples of 15 or 20 fruit. One set of samples was uninoculated. To give consistently high infection, a second set was inoculated with spores of the brown rot fungus (*Monilinia fructicola* (Wint.) Honey) and a third set was inoculated with spores of the Rhizopus rot fungus (*Rhizopus stolonifer* (Ehr. ex Fr.) Vuill. in a manner previously described (5). About 2 hours after inoculation the fruit was hydrocooled by submersion for 15 minutes in ice water or in an ice water solution of 0.1% Dowicide A (97% sodium *o*-phenylphenoxide) containing 0.1% trisodium phosphate as an alkaline buffer.

The hydrocooled samples were immediately placed in open 4-qt. tills at holding temperatures of 40°, 45°, 50°, 55°, or 60°F, left 3 days, and removed to a 75° room for 3 or 6 days. Decayed peaches were counted and removed after the holding period, and after 3 and 6 days at 75°, and the cumulative decay percentage was computed.

The firmness of 20 comparable fruit was measured with a Magness-Taylor pressure tester with a $\frac{5}{16}$-in. plunger (2), at the start of each experiment, on removal from the holding temperatures, and after a subsequent 3 days at 75°F.

This experiment was performed 11 times during the 1957 season.

RESULTS.—The average peach temperature was 76.0°F before hydrocooling and 53.4° afterward. Water temperature during hydrocooling averaged 41.0°.

During the 3-day holding period at 60°F, inoculated peaches hydrocooled in plain ice water developed 40% Rhizopus rot and 2% brown rot. When the hydrocooling solution contained Dowicide A, the respective decays were 5 and 1%. During the 55° holding period, inoculated peaches developed 11% Rhizopus rot after hydrocooling in plain ice water, as compared with 3% after hydrocooling in a Dowicide A solution. No brown rot developed on peaches held at 55°, and neither decay developed on peaches held at 50° or lower.

After 3 days at 75°F, the percentage of brown rot in inoculated peaches hydrocooled in plain ice water was consistently less in fruit held at 40° than in fruit held at higher temperatures (Table 1). When the previous holding temperature was 40° or 45° Rhizopus rot was considerably lower than when higher holding temperatures were used. Percentage of reduction of both types of decay was usually high when Dowicide A was added to the hydrocooling solution. The percentage reduction of both decays by Dowicide A was lowest when fruit was held at 60° previous to the 3 days at 75°.

After 6 days at 75°F, the percentage of brown rot in inoculated peaches hydrocooled in plain ice water was not affected by the previous holding temperature (Table 1). Rhizopus rot of such peaches was somewhat lower when the previous holding temperature was 40° or 45° than when higher temperatures were used. Both decays were slightly reduced when Dowi-

TABLE 1.—*Cumulative decay of peaches hydrocooled in ice water with and without Dowicide A, held 3 days at various temperatures, and subsequently held at 75°F*

Inoculation status and type of decay	Holding temperature (°F)	% decay after 3 days at 75°F		% decay reduced by	% decay after 6 days at 75°F		% decay reduced by
		Ice water	Dowicide A	Dowicide A	Ice water	Dowicide A	Dowicide A
Inoculated[a]							
Brown rot	40	39	7	83	84	62	26
	45	55	16	71	84	63	25
	50	64	11	82	83	69	17
	55	81	25	69	89	70	22
	60	71	39	45	77	71	8
Rhizopus rot	40	50	26	49	71	49	32
	45	61	35	43	67	51	24
	50	77	37	53	84	63	26
	55	78	42	47	81	56	31
	60	84	64	24	86	73	15
Not inoculated							
Both rots	40	13	4	73	48	37	23
	45	21	4	82	50	41	18
	50	31	10	67	58	45	22
	55	23	9	58	46	31	31
	60	34	23	32	54	40	26
LSD Brown rot 5%		13.5	13.5		16.5	16.5	
1%		17.9	17.9		21.8	21.8	
LSD Rhizopus rot 5%		14.6	14.6		13.9	13.9	
1%		19.3	19.3		18.5	18.5	
LSD Not inoculated 5%		12.5	12.5		13.2	13.2	
1%		16.8	16.8		17.6	17.6	

[a] Peaches were inoculated about 2 hours before being hydrocooled.

cide A was added to the hydrocooling water.

Decay of uninoculated fruit after 3 days at 75°F was lowest in peaches held previously at 40°, and highest in those held previously at 60°. After 6 days at 75°, the previous holding temperatures no longer affected the decay of peaches.

The average firmness of the peaches was originally 12.9 lb. and fell to 11.8, 9.5 or 4.7 lb., respectively, after 3 days at 40°, 50°, or 60°F. In every replication the peaches were fully ripe after 3 days at 75°.

Discussion and conclusions.—The present study shows the effect of various simulated transit temperatures on the subsequent decay of peaches. After 3 days at 75°F peaches transferred from a simulated transit temperature of 40° had less brown or Rhizopus rot than those transferred from 60°. This could be attributed to a) the reduction of the growth rate of the pathogens while the peaches were at 40°; b) the longer time needed for peaches held at 40° than for those held at 60° to warm to temperatures suitable for rapid infection; c) the greater firmness of peaches at the time of transfer to 75° after holding at 40° than after holding at 60°; or d) some other factor associated with a low temperature holding period.

After 6 days at 75°F the percentage of brown rot in inoculated fruit was about the same in all lots regardless of the previous holding temperature. Evidently a period of low temperature did not reduce the ability of the brown rot pathogen to decay peaches when subsequently transferred to 75°. On the other hand, decay in Rhizopus-inoculated fruit held previously at 40° or 45° remained less than in those held at higher simulated transit temperatures. Rhizopus rot in peaches held previously at 40° was even less after 6 days at 75° than after 3 days at 75° following a 60° holding temperature. It seems improbable that Rhizopus rot, which normally develops rapidly at 75°, would show a difference in decay percentage after 6 days that could be attributed to a delay in onset of infection when the fruit was placed at 75° or while it was at low temperatures. The reduction of Rhizopus rot of peaches by a previous period of low temperature may therefore be due to some factor which has a lasting effect on either the virulence of the pathogen or the conditions for infection.

Dowicide A has been reported to reduce both brown and Rhizopus rot of peaches, either when applied as a postharvest spray (5) or when added to hydrocooling water (1, 3, 4). In the present tests, hydrocooling peaches in Dowicide A solution reduced decay in all cases below that of comparable peaches hydrocooled in plain ice water. The effectiveness of a fungicidal treatment in reducing decay below that of untreated peaches is undoubtedly influenced by many factors. This study shows that decay reduction by Dowicide A was less when peaches were held at 60°F before transfer to 75° than when they were held at lower temperatures.

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SOME STUDIES IN THE USE OF IONIZING RADIATION TO CONTROL BOTRYTIS ROT IN TABLE GRAPES AND STRAWBERRIES[1]

K. E. Nelson, E. C. Maxie, and W. Eukel[2]

SUMMARY

Freshly harvested table grapes were irradiated in 2-lb. consumer packages with different doses of ionizing radiation (electrons). After 3 and 9 days at 3–4°C, Thompson Seedless grapes dosed with 4×10^5 rep turned slightly brown and had a faint "cooked" odor and flavor. At 8×10^5 rep these symptoms were more pronounced, and berry cracking was evident. Injury was less apparent in Tokay and Emperor grapes, owing to the masking effect of the skin pigment. Thompson Seedless grapes that had been 6 weeks in cold storage before treatment showed some injury after being irradiated with 2×10^5 rep. Shasta strawberries irradiated in monolayers showed no injury at 2×10^5 rep; at 4×10^5 their interiors had a water-soaked appearance as well as a "cooked" odor and were off-flavor, and at 8×10^5 the berries exuded juice. Mycelial transfers of *B. cinerea* on grape-juice agar were irradiated with different doses, and held at 3–4°C. A dose of 1×10^5 delayed growth 5–7 days, 2×10^5 delayed growth 10–14 days, 4×10^5 resulted in slight growth, which stopped, and 8×10^5 prevented subsequent growth. Cultures irradiated with 4×10^5 rep made no growth after transfer to unirradiated media. The rate of spread of botrytis rot among grape berries and strawberries was markedly reduced at doses of 1×10^5 and 2×10^5 rep.

INTRODUCTION.—Attempts to sterilize with ionizing radiation date back to early researches on its bactericidal properties (6), soon after Roentgen's discovery of the X ray (7) and Becquerel's discovery of natural radioactivity (1). A strong interest in applying irradiation-sterilization to the preservation of foods began with the development of electron generators, in the early 1940's. This interest was given impetus by the report of Brasch and Huber (3), which demonstrated the possibility of using such equipment with foodstuffs. Hannan (4) has reviewed the technical problems involved in irradiation-sterilization of foods. He emphasized that complete sterilization requires doses far beyond the tolerance of most fruits and vegetables. An alternative is to use low doses, with the objective of temporarily inhibiting the growth of pathogens. Beraha et al (2) demonstrated the significance of this alternative. Using gamma rays, they were able to show marked suppression of growth of several common decay organisms in infected fruits at doses that appeared to cause little or no apparent injury to the commodity. These results indicated the possibility of extending the shelf-life of fresh fruits with postharvest irradiation.

One of the most promising applications of irradiation in this field appeared to be in the control of decay caused by *Botrytis cinerea* Pers. on table grapes and strawberries. Not only is this fungus the primary cause of decay in these fruits (9) but it is impossible to eradicate infections with chemical fungicides without injuring the fruit excessively (5). The penetrating power of gamma rays is well known, and Beraha et al (2) demonstrated that these rays reach decay organisms in areas of fruits not now accessible to chemicals. However, the use of gamma rays requires massive fixed installations to protect personnel. The source of the rays is usually spent fuel rods or Cobalt-60. If beta particles would effectively penetrate infected fruit and produce a fungicidal or even a fungistatic effect without fruit injury, then this type of radiation could have strong possibilities as a postharvest treatment. This treatment would have the advantage of more flexibility and a higher dose rate, i.e., shorter exposure period, for comparable capital invested.

In this investigation beta particles were used to determine: 1) symptoms of irradiation injury on table grapes and strawberries and the relationship of these symptoms to dose level; 2) the growth rate of *B. cinerea* in pure culture after irradiation treatment at different dose levels; and 3) the rate of spread of botrytis rot from diseased to healthy fruit after various irradiation doses.

MATERIALS AND METHODS.—An ARCO Model 1 Mark 1 electron linear accelerator was used as the source of radiation (Fig. 1). It was capable of producing a 1.0-kw beam of 8 Mev electrons; however, the accelerator output was reduced to one sixth of capacity to permit accurate dose measurements at the low dose range used, i.e., about 2×10^4 rep per minute. The conveyor was an aluminum turntable 4 ft. in diameter rotating at 10 rpm. Combined errors of dose from batch to batch resulting from differences in time, accelerator output, and position of the sample on the turntable did not exceed 4%.

The dose was determined by a change in optical density of cobalt glass as measured with a Bausch and Lomb colorimeter No. 33-29-40. Estimated error in colorimeter and glass combined was within 5%. Only the central part of the beam was used, so that areal uniformity of dose was kept within 10%. Areal variation was negligible for the fungus culture in Petri dishes, where only a fraction of an inch of the central part of the beam was used. When uniform fruit was

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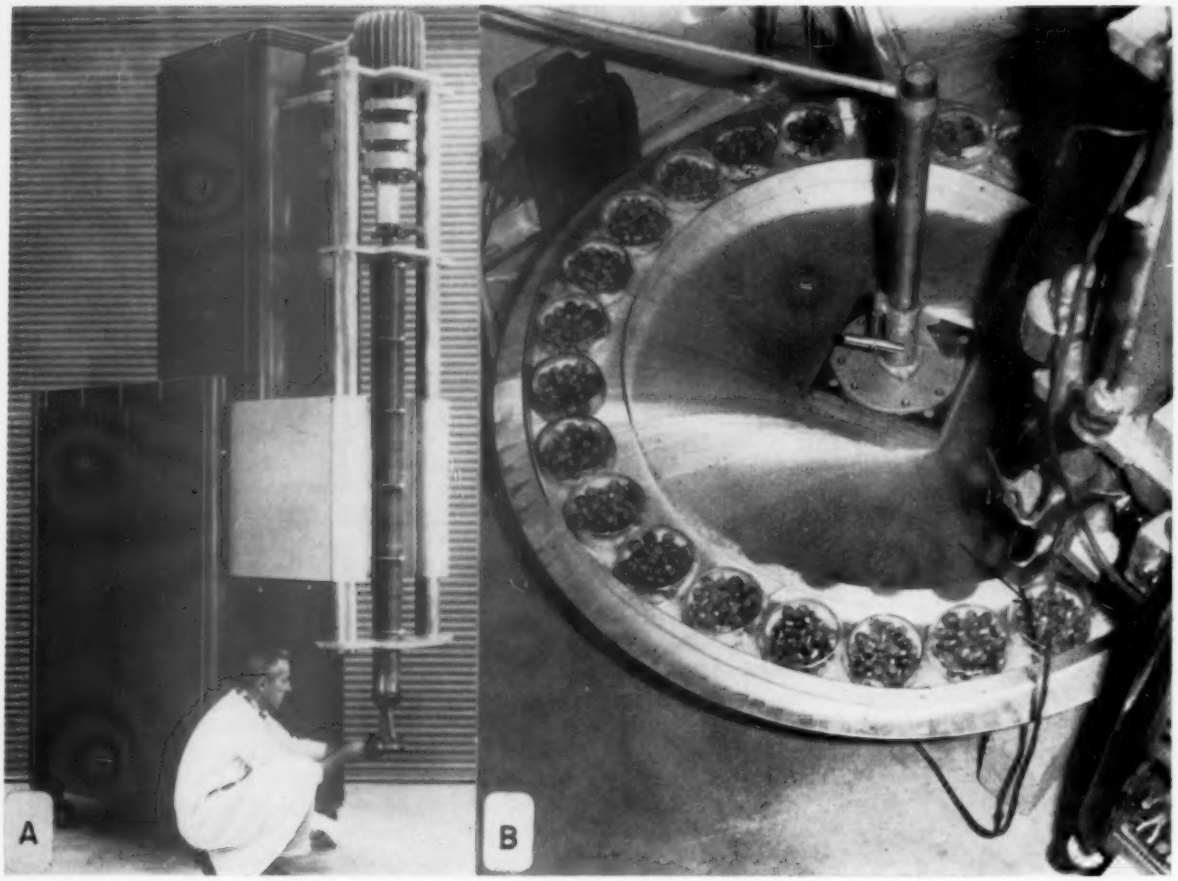

Fig. 1. Radiation facility. **A)** Over-all view of an ARCO Model 1 Mark 1 electron linear accelerator, showing operator adjusting beam scanning window at the lower end of electron gun. **B)** Aluminum turntable in place under electron gun. Fruit passes repeatedly through beam area (arrow).

treated in monolayers less than 2 cm thick, the dose variation from top to bottom of the berries did not exceed ±10%. When grapes were packed in 2-lb. consumer packages (6×4×2½ in.) the variation was 20–25%; however, for the following tests the packages were irradiated from both top and bottom, which reduced variation below 10%.

Mature Tokay, Emperor, and girdled Thompson Seedless grapes and Shasta strawberries were used for the fruit tests. Small grape clusters of 3–10 berries were packed in 2-lb. consumer packages for tests to evaluate injury to stems and berries. In all other tests the fruit was irradiated in monolayers—the grape berries in glass stacking dishes and the strawberries in 1-lb. plastic baskets. Monolayered fruit was irradiated from the top only.

In infected fruit studies, berries were inoculated by spraying with a conidial suspension of *B. cinerea*, and then incubated 24 hours in a moist chamber at 20°C. Infected and healthy strawberries were intermingled in closely packed monolayers. Infected Tokay berries were intermingled with healthy Emperor berries in monolayers so that each healthy berry was in contact with two infected berries. Each stacking dish was covered with acetate film to prevent outside contamination while permitting visibility and escape of water vapor. All fruit was cooled to 4°C immediately after packing, irradiated within 4 hours, and then held at 3–4°C until evaluated.

Six dose levels were used on strawberries: 0; 5×10^4; 1×10^5; 2×10^5; 4×10^5; and 8×10^5 rep. The berries were held 10 days at 5°C in open cups. Observations for injury symptoms were made daily.

Pure cultures of *B. cinerea* were used for fungus growth rate studies. Three-mm disks of nonsporulating *Botrytis* mycelium on agar were transferred to the center of Petri dishes containing 25 ml of grape-juice-agar media. The dishes were held 18 hours at 20°C, irradiated, held 4 hours at 20°C, and then held at 3–4°C while being periodically measured.

EXPERIMENTAL RESULTS.—*Injury symptoms on fruit.*—The grapes irradiated in consumer packages were evaluated for injury on the third and ninth days after treatment (Table 1). The Thompson Seedless berries showed no symptoms of injury at dose levels of 2×10^5 rep and lower, both on the third and ninth days. The pedicels were slightly brown; the laterals and main stems appeared unaffected. The berries and stems of the 4×10^5 rep lot showed distinct symptoms of injury

TABLE 1.—*The effect of radiation (electrons) on the appearance and flavor of Thompson Seedless and Tokay grapes held at 3–4°C after treatment*[a]

Variety and Dose	Days following treatment			
	3		9	
	Berries	Stems	Berries	Stems
Thompson Seedless				
None	NC[b]	NC	NC	SlBr[b]
5×10^4 rep	NC[b]	NC	NC	SlBr
1×10^5 rep	NC[b]	NC	NC	SlBr
2×10^5 rep	NC[b]	SlBr	NC	SlBr
4×10^5 rep	SlBr SlCr[b]	ModBr[b]	50% Br SlCr SlCO&F[b]	AllBr
8×10^5	AllBr[b] 25% Cr	MoBr[b]	AllBr 30% Cr CO&F	AllBr
Tokay				
None	NC	NC	NC	SlBr
5×10^4 rep	NC	NC	NC	SlBr
1×10^5 rep	NC	NC	NC	SlBr
2×10^5 rep	NC	NC	NC	SlBr
4×10^5	NC	ModBr	SlBr SlCO&F	MoBr
8×10^5 rep	SlBr	ModBr	ModBr SlCO&F	AllBr

[a] No discernible change in appearance or flavor at all dose levels immediately after treatment.

[b] NC, no change; SlBr, slight browning; ModBr, moderate browning; SlCr, slight cracking; MoBr, mostly brown; CO&F, cooked odor and flavor; AllBr, all brown.

on the third day, which were more pronounced on the ninth. The fruit of the 8×10^5 rep lot was very brown, with a distinct cooked odor and flavor. Cracking was very pronounced.

There were no symptoms of injury on Tokay grapes treated with 2×10^5 rep. Even fruit treated with 4×10^5 rep showed no injury on the third day. However, the typical brown, cooked injury symptoms were evident on the ninth day; in the 8×10^5 rep lot they were very pronounced on both days. No cracking was noted in this variety.

It appears that the pigment of Tokay grapes tends to mask symptoms of injury (Fig. 2). Typical berries from the foregoing tests were photographed on the fourteenth day after treatment. They had appeared the same as on the ninth day. The unpigmented Tokays (B) and Thompson Seedless berries (A) show the same injury response; the heavily pigmented Tokay berries (C) manifest no response although the cooked flavor and odor was present in fruit treated with 4×10^5 rep.

In later tests, Thompson Seedless grapes that, before treatment, had been 6 weeks in storage showed browning symptoms at the 2×10^5 rep level 1 week after treatment, indicating that the fruit becomes more susceptible to injury as it ages. In contrast, unstored Emperor berries showed no injury at all from dose levels up to and including 5×10^5 rep. However, the stems showed injury from 4×10^5 rep.

Immediately after irradiation, the Shasta strawberries that had received 8×10^5 rep exuded minute,

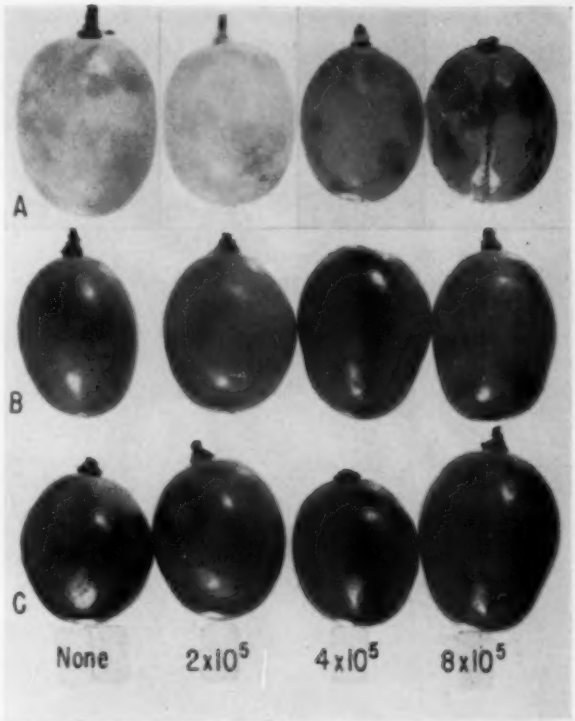

Fig. 2. Radiation injury symptoms on table grapes 14 days after treatment and held at 3°C. **A)** Thompson Seedless berries treated with: no radiation, 2×10^5 rep, 4×10^5 rep, and 8×10^5 rep, respectively. **B)** Tokay berries, with practically no pigment, treated likewise. **C)** Heavily pigmented Tokay berries treated in the same manner.

red droplets of juice. The interior of the berries had a brownish, water-soaked appearance. The 4×10^5 lots showed no surface exudate, but had internal symptoms similar to those observed in the 8×10^5 lots. Both lots had a "cooked" odor and a very pronounced off-flavor. No evidence of physical injury, off-flavor, or odor was noted in lots subjected to 2×10^5 rep or less, which is in agreement with the findings of Beraha et al (2). All lots showed no further changes for 4 days. On the

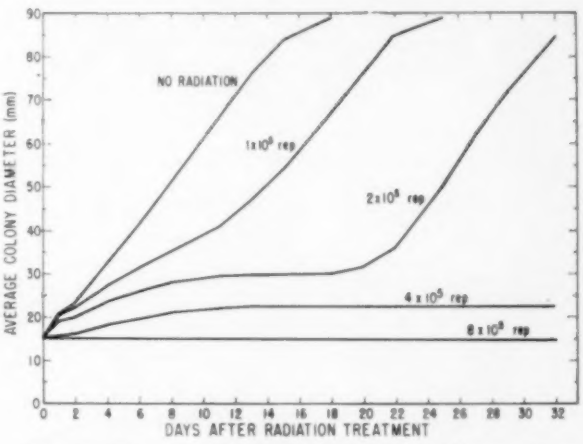

Fig. 3. The effect of ionizing radiation (electrons) on the subsequent growth of *Botrytis cinerea* in pure culture at 3–4°C.

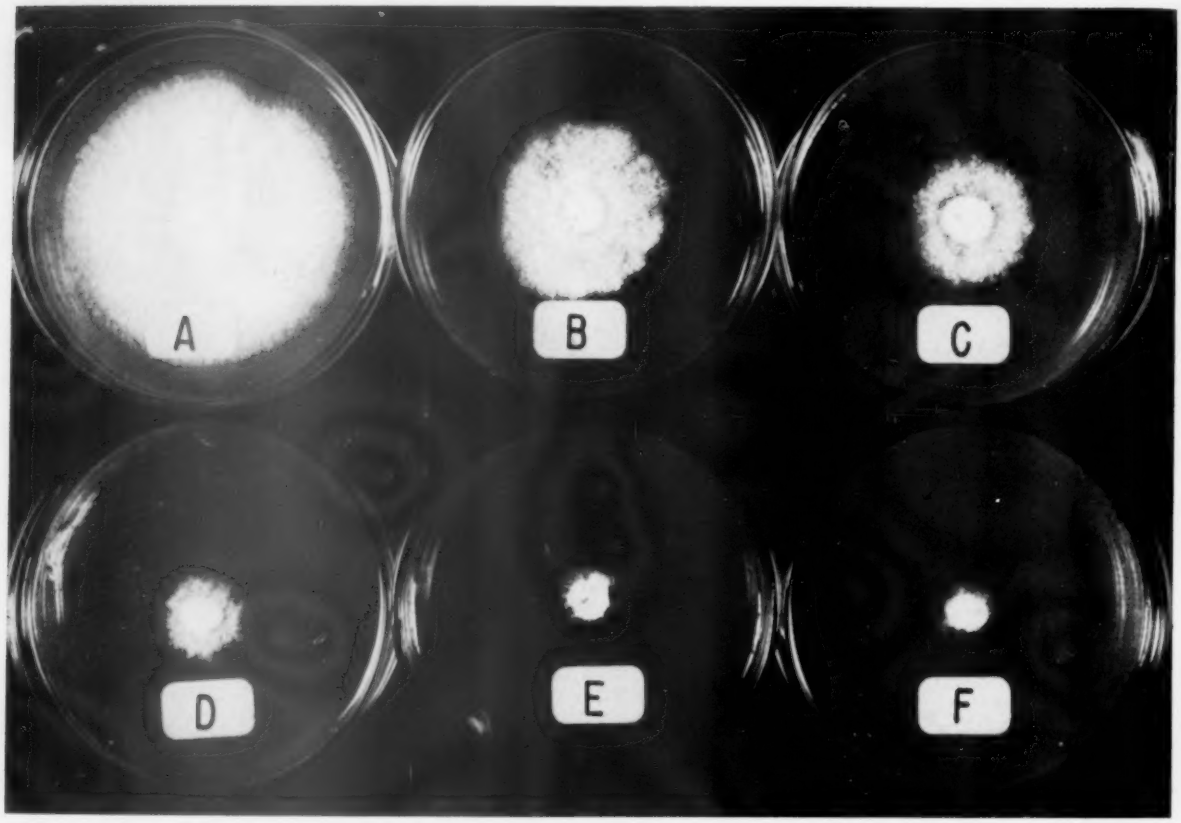

Fig. 4. Botrytis cultures 11 days after radiation treatment and held at 3–4°C. **A)** No radiation treatment; **B)** 1×10^5 rep; **C)** 2×10^5 rep; **D)** 4×10^5 rep; **E)** 8×10^5 rep; **F)** 12×10^5 rep.

fifth day, the 8×10^5 lot collapsed into soggy masses. The red pigments were bleached out, leaving a water-soaked appearance. At the end of 10 days, extensive bleaching of red pigments had occurred in the 4×10^5 lots, though these berries did not collapse. No injury symptoms were noted at any time in the lots receiving 2×10^5 or less, and there were no detectable off-flavors or odors. However, critical consumer-acceptance taste tests would be necessary to determine the relationship of flavor and texture to dose level.

Irradiation effect on growth of B. cinerea in pure culture.—The diameter of the irradiated cultures of *B. cinerea* was determined at about 2-day intervals for 32 days. The average diameters of 5 cultures at each dose level are shown in Fig. 3. Unirradiated cultures grew at a constant rate until the edges approached the sides of the Petri dishes, on about the sixteenth day. Cultures irradiated at 1×10^5 rep, however, grew appreciably more slowly for 11 days and thereafter grew nearly as rapidly as unirradiated cultures. Cultures irradiated with 2×10^5 rep grew very slowly for 11 days, stopped until the eighteenth day, when growth was resumed, and by the twenty-second day reached the same rate of growth as that of unirradiated lots. Cultures irradiated with 4×10^5 rep showed very slight growth for about 12 days, then stopped, and did not resume growth. Cultures irradiated with 8×10^5 rep

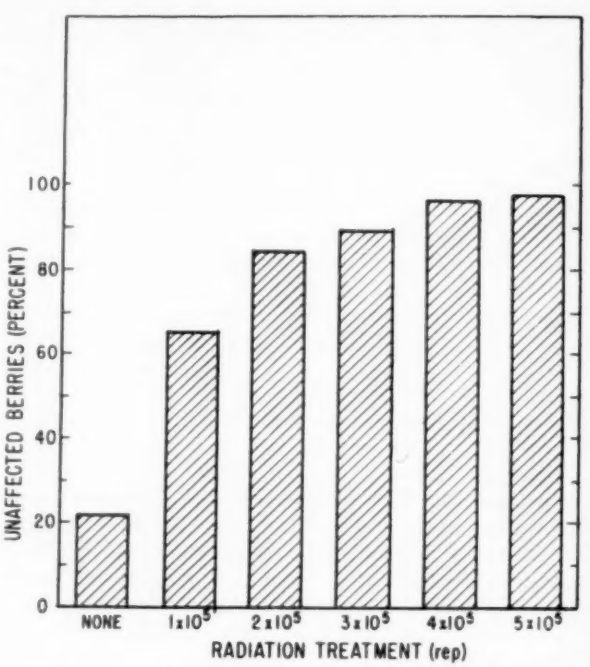

Fig. 5. Irradiating healthy and Botrytis-affected grape berries in contact; proportion of healthy berries still unaffected after 14 days at 3–4°C.

showed no growth after treatment.

Density of the mycelium was likewise inversely correlated with dose. This is apparent in Fig. 4, a photograph of representative cultures the eleventh day after treatment. The dense center of each culture is the original transfer disk. In both area and density, the amount of mycelial growth from the center disk is progressively less with greater doses. The small amount of mycelium around the central disk in the cultures irradiated at 8×10^5 and 12×10^5 rep denotes the growth made between time of the transfer and time of treatment.

On the thirty-second day, mass transfers were made from the cultures to sterile unirradiated media. No viable transfers were secured from the cultures irradiated at 4×10^5 rep or more. All other transfers grew normally and at the same rate.

Effect of irradiation on rate of contact infection between fruits.—The number of botrytis-affected Emperor berries in the stacking dishes was determined on the fourteenth day after treatment. The proportion still unaffected at each dose level (average of 4 dishes) is shown in Fig. 5. It is apparent that a dose of 1×10^5 rep markedly retarded the rate of contact infection, and that doses above 2×10^5 gave very little additional benefit.

The extent of signs of the fungus on the originally infected Tokay berries was inversely related to the dose (Fig. 6), particularly in the lower dose range. The mycelial ridges almost enveloped the infected berries (Fig. 6-AC), whereas in the higher ranges the fungus had made little to no progress by the fourteenth day (Fig. 6-BFG).

The untreated strawberries showed extensive Botrytis growth by the tenth day. There was extensive spreading of mold from laboratory-infected to sound berries. Many berries not in contact with infected berries were covered with mold, apparently from field infections. The 5×10^4 lot showed little mycelial growth, but the fungus sporulated profusely. There was no evidence of mold growth in either the 1×10^5 and 2×10^5 lot. The laboratory-infected berries had a

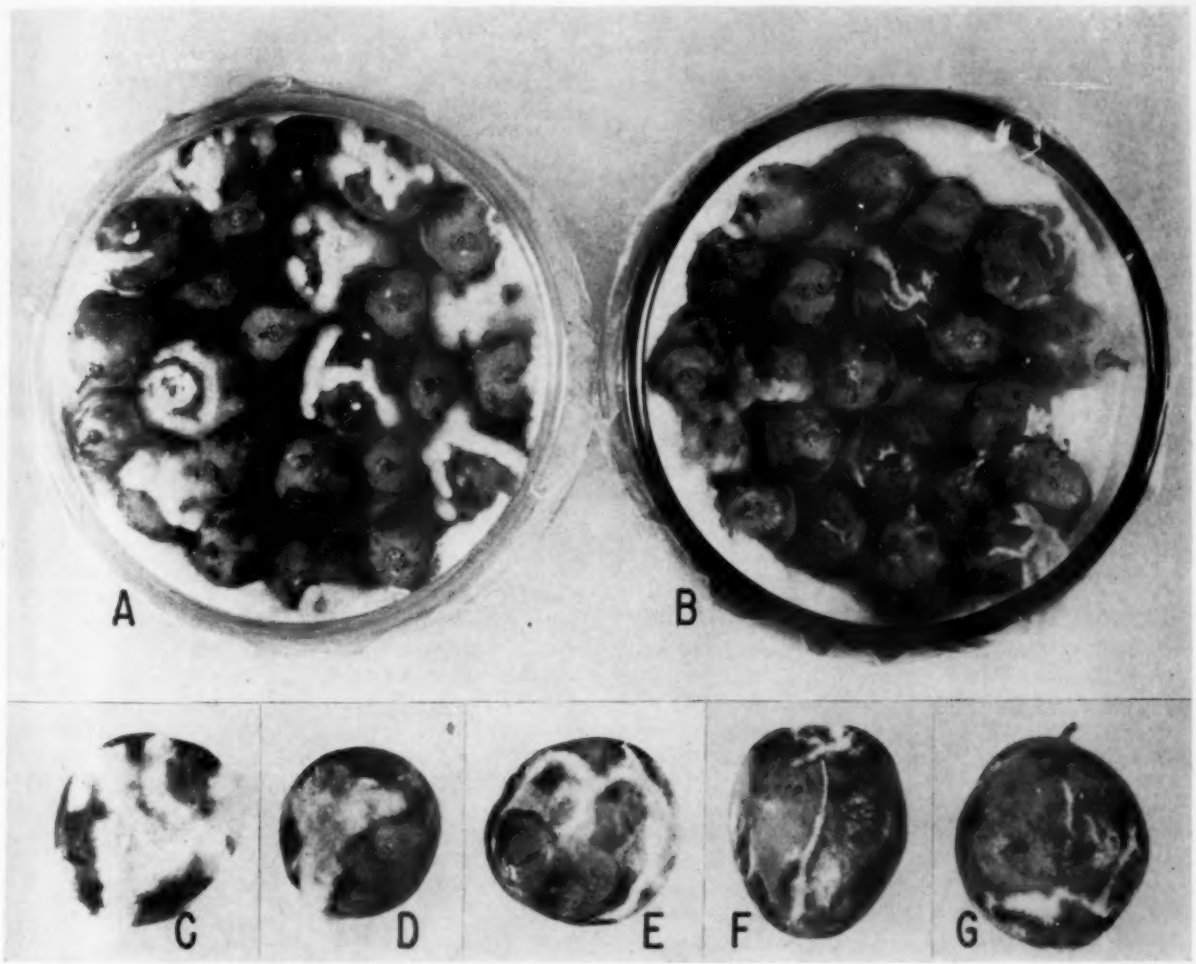

Fig. 6. A-B) Acetate-film-covered stacking dishes containing healthy and botrytis-affected grape berries in contact after 14 days at 3-4°C: **A)** No initial radiation treatment; **B)** initially irradiated with 4×10^5 rep. **C-G)** berries infected at time of radiation treatment, held at 3-4°C, and photographed 14 days later: **C)** no radiation; **D)** 1×10^5 rep; **E)** 2×10^5 rep; **F)** 3×10^5 rep; **G)** 4×10^5 rep.

mummied appearance. There was no evidence of transfer of mold from infected to adjacent berries.

DISCUSSION.—Hannan's (4) conclusion that decay control is not practical in most fruits and vegetables by sterilization is supported by the results of this investigation with grapes and strawberries infected with *B. cinerea*. However, there appears to be a range of doses that has marked fungistatic effect without producing apparent injury. The effects are temporary, but of sufficient magnitude and duration to offer possibilities of increasing the postharvest life of the fruit. If an arbitrary level of growth of 50 mm is chosen, it is evident in Fig. 3 that cultures treated with 1×10^5 rep required nearly twice as long to reach this level as did those not treated, and cultures treated with 2×10^5 rep required over 3 times as long. It is interesting that, at these levels, doubling the dose (from 1×10^5 to 2×10^5 rep) nearly doubled the period of fungistatic effect.

It appears that gamma rays and electrons at the same rep dose produce about the same fungistatic and fungicidal action. Beraha et al (2), using gamma rays, irradiated grapes inoculated with *B. cinerea* and found that the fungistatic effect was inversely proportional to the dose in the range of 5×10^4 to 3×10^5 rep. At doses of 5×10^5 rep and higher, no rot developed within 10 days of treatment (the incubation temperature was not reported). In the present study, fungistatic action was evident at 1×10^5 and 2×10^5 rep (Fig. 3). Unfortunately, cultures were not treated with 5×10^4 and 3×10^5 rep. A dose of 4×10^5 rep was apparently fungicidal, since growth stopped after treatment and transfers to fresh media were not viable.

Electrons have poor penetrating power in comparison with gamma rays. This deficiency could be serious in irradiating whole packages of fruit. At 12.2 Mev, the received dose drops off to 50% in water at a depth of 4.25 cm (8). However, by irradiating from both top and bottom the depth can be increased to 8.5 cm and still keep dose variation within 10%. This depth is greater than that of baskets of strawberries or 2-lb. consumer units of grapes. It is possible to irradiate this size of package with this energy, assuming that electron dose drops off with depth in these fruits at about the same rate that it does in water. Dose would vary much more from fruit to fruit and from one part of a fruit to another than it would in water, because of air voids between the berries.

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FUSARIUM BLIGHT OF BIRDSFOOT TREFOIL[1]

Richard E. Ford

SUMMARY

Diagnostic symptoms of a leaf and stem blight of birdsfoot trefoil (*Lotus corniculatus*) are bleaching and drooping of stem tips, followed by death of the affected shoots. *Fusarium roseum* was isolated consistently from plants showing these symptoms. Except for a leaf scorch, plants inoculated with pure cultures of this fungus produced symptoms the same as those observed on plants in the field. *F. roseum* was reisolated from each plant infected under controlled conditions. Narragansett and DuPuits alfalfa (*Medicago sativa*) and Pennscott red clover (*Trifolium pratense*) were inoculated with *F. roseum* isolated from birdsfoot trefoil. Necrotic lesions formed on the leaves of each plant and *F. roseum* was reisolated. In addition to *L. corniculatus*, 6 other *Lotus* spp. were found susceptible. *L. corniculatus* and *L. corniculatus* var. *japonicus* seedlings 1–3 weeks old were not susceptible to a root rot caused by *F. roseum;* however, 3 related species (*L. arenarius, L. divaricatus,* and *L. maroccanus*) exhibited severe root rot 3 weeks after transplantation into steam-sterilized soil infested with *F. roseum*. Optimum temperature for disease development on birdsfoot trefoil foliage at 100% relative humidity was near 82°F. Free water was required on the foliage for at least 10 hours for severe disease to develop. Cultures fragmented in a blender were more infective than suspensions of about 13 spores per 470× field of the microscope. Wounding at inoculation resulted in increased disease severity, but was not essential to ingress.

INTRODUCTION.—Birdsfoot trefoil (*Lotus corniculatus* L.) is relatively free from foliage diseases, especially when compared with such crops as alfalfa (*Medicago sativa* L.) and red clover (*Trifolium repens* L.), each of which has at least 1 or 2 diseases that cause appreciable annual losses (17). Although several parasitic fungi have been reported on birdsfoot trefoil (20), copper spot, caused by *Stemphylium loti* Graham, is apparently the only serious foliage disease of this plant in the United States. Crop losses, however, have never been estimated at more than 3% (16).

The increased use of birdsfoot trefoil presupposes an increase in the importance of diseases of that crop. It is not surprising, therefore, that a stem and leaf blight first observed in New York during the summer of 1957 apparently has not been described previously except in a preliminary report (10) on the work described herein. This paper reports the results of studies of the symptoms of this disease and the pathogenicity of the causal agent.

SYMPTOMS.—In the summer of 1957, a few blighted plants of a bleached tan stood out against the dark-green background of vigorously growing seedlings in a stand of birdsfoot trefoil near Ithaca, N. Y. The severity of blight varied. In some plants the top ½–1 in. of the shoot was blighted; in others, the whole stem was blighted. A few plants that showed apical yellowing were marked, and later observations confirmed that the blight had begun as an apical yellowing, followed by a bleaching of blighted tissues. In some plants, the blight progressed from the apices of shoots to the crowns of the plants, whereas in others it became arrested, and only stem tips dried and collapsed. A general characteristic of this blight was the drooping of the apices of all blighted stems (Fig. 1-A). These symptoms were observed in seedling stands or in 2–5-year-old stands in 5 widely separated counties in New York during July and August of 1957.

Symptoms observed were suggestive of the foliage blight caused by *Rhizoctonia solani* Kuehn, which has been destructive in the southeastern United States (1, 2, 3, 4) and in Iowa (5, 6, 7). Rhizoctonia blight appears to be most destructive on established stands (1), and leaves of big trefoil (*Lotus uliginosus* Schkuhr) are killed long before there is noticeable stem injury (21). Moreover, sclerotia of *R. solani* are frequently found on plants blighted by that fungus (2). The blight reported here is especially severe in seedling stands, and often affects stems before it affects leaves; further, no sclerotia could be found on diseased plants. For these reasons, this blight was considered to be distinct from Rhizoctonia blight, and studies were made to determine its etiology.

PROOF OF PATHOGENICITY OF FUSARIUM ROSEUM.—*Isolation of fungi associated with blight of birdsfoot trefoil.*—Samples of diseased tissues from 79 plants were collected. Small sections of blighted or yellowed stems and leaves were surface-sterilized by immersion in 20% commercial Clorox (5.25% sodium hypochlorite) for 1–3 minutes. Five to 7 sections were placed on hardened 2% PDA (potato-dextrose agar)[2] in each of 107 Petri dishes. The culture medium was acidified by adding 50% lactic acid at the rate of 1 drop per dish. In the event that the organism associated with blight of birdsfoot trefoil might not grow readily from diseased tissue onto PDA, 10 dishes each

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A portion of a thesis presented to the Graduate School, Cornell University, in partial fullfillment of the requirements for an M.S. degree. The author expresses his sincere gratitude to Dr. D. A. Roberts for his guidance. Thanks are also due Drs. R. R. Seaney, C. C. Lowe, D. G. Clark, and G. C. Kent for critical reading of the manuscript.

[2] Supernatant from 200 g cooked potatoes, 20 g dextrose, 20 g agar agar, and distilled water to make a total volume of 1 liter.

Fig. 1. A) Stem and leaf blight of *Lotus corniculatus*. Left, diseased shoot; right, healthy shoots. **B)** Aerial mycelium of *Fusarium roseum* on blighted leaves of *Lotus corniculatus* 2 days after inoculation. This plant had been in continuous light of 250 ft-c intensity in a chamber kept at 80-84°F and at 100% R.H.

of corn-meal agar,[3] oatmeal agar,[4] and Lima-bean agar[5] were used as culture media in the first series of isolations.

Fusarium roseum (Lk.) emend. Snyd. & Hans.[6] grew from 179 tissue sections in 65 dishes; transfers were made and the fungus was grown in pure culture. This fungus developed from yellowed as well as from blighted tissues, and grew as well on PDA as on any of the other media used. An *Alternaria* sp. was isolated frequently, but only from blighted tissue. In other experiments, a nonpathogenic *Alternaria* sp. indistinguishable from it, had been frequently found to be associated with necroses incited by *Stemphylium loti*. A *Rhizoctonia* sp. was isolated from the crown of an old plant and from 4 blighted stem or leaf sections. Because the *Alternaria* sp. is probably nonpathogenic, and because the *Rhizoctonia* sp. was only rarely isolated, it appeared that *F. roseum* was the primary causal agent of blight of birdsfoot trefoil. Nevertheless, each of these fungi was grown in pure culture and was used in pathogenicity tests.

Two distinct strains of *F. roseum* were isolated from blighted shoots of birdsfoot trefoil. One of these, herein termed the red strain, was characterized by the production of a dark pink to red pigment in the mycelium and/or spore masses, and by an appressed habit of growth; the other strain, termed the brown strain, produced a brown pigment in the mycelium and produced more aerial mycelium than the red one. Further, microconidia were produced by the brown strain, but these were never observed in cultures of the red strain.

Inoculation of birdsfoot trefoil and other plants with Fusarium roseum and other fungi.—Inocula were prepared from 1-week-old PDA cultures that had been kept in the laboratory at about 70°F. Each inoculum was prepared by mixing 1 Petri dish culture with

[3] Twenty g corn-meal, 20 g agar agar, and distilled water to make a total volume of 1 liter.

[4] Supernatant from 50 g oatmeal, 20 g agar agar, and distilled water to make a total volume of 1 liter.

[5] Supernatant from 100 g frozen Lima beans ground in a Waring Blendor, 20 g agar agar, and distilled water to make a total volume of 1 liter.

[6] Identified with the assistance of O. C. Maloy; confirmed by W. C. Snyder.

about 40 ml of distilled water, fragmenting in a Waring Blendor, and straining through cheesecloth. One drop of Tween 20[7] was added to each 500 ml of inoculum. Inocula were atomized onto plants until run-off. Test plants were 3–9-month-old greenhouse-grown Empire and Viking birdsfoot trefoil plants that had been cut back to the crown 3 weeks before inoculation. In 1 experiment, inoculated plants were kept in a chamber at 70–78°F and 100% RH for 2 days; in 2 other tests, inoculated plants were kept at 80–84°F. During this time, plants received about 250 ft-c illumination continuously. Two days after inoculation the plants were moved to the greenhouse, where the temperature range was 66–78°F. Seventy-nine plants were used in 3 experiments.

Stem and leaf blight symptoms typical of those observed in the field developed on birdsfoot trefoil plants 4 days after they had been inoculated with *F. roseum*, regardless of which strain of the fungus had been used as inoculum. The red strain or the brown strain of this fungus was reisolated from every infected plant that had been inoculated. No organism could be reisolated from green tissues of inoculated plants. Disease was more severe and developed more rapidly on plants incubated at 82°F than at 74°F. Wefts of aerial mycelium developed on plants kept at the higher temperature (Fig. 1-B). In plants inoculated under controlled conditions, both strains of *F. roseum* caused a leaf scorch besides the characteristic blight and drooping of shoots. The scorch symptom was not a part of the syndrome observed in the field, probably because RH was not 100% continuously for 2 days following inoculation.

No symptoms developed on any of the plants that had been inoculated with an *Alternaria* sp. or a *Rhizoctonia* sp. These tests provide evidence that *F. roseum* is the causal agent of the stem and leaf blight observed. The *Rhizoctonia* sp. studied might be pathogenic to roots and crowns of birdsfoot trefoil plants, but under the conditions on this experiment it was incapable of causing a blight of aboveground parts of the plant.

In 1 experiment, 10 plants each of Narragansett and DuPuits alfalfa and of Pennscott red clover were inoculated as before with each of the 2 strains of *F. roseum*, and were kept 2 days at 100% RH and 82°F before they were moved to the greenhouse. In another experiment, 38 plants of 18 lines of *Lotus* spp. were inoculated with either the red or the brown strain of *F. roseum*. Eleven of the lines inoculated were of *L. corniculatus* L., 2 were of *L. arenarius* Brot., and 1 was from each of the species *L. rigidus* Greene, *L. australis* Andr., *L. maroccanus* Ball, *L. peregrinus* L., and *L. divaricatus* Boiss. All inoculated plants became diseased, and *F. roseum* was reisolated from each diseased alfalfa and red clover plant, and from representative samples of the *Lotus* spp. On alfalfa plants, symptoms were similar to those observed on birdsfoot trefoil. On red clover, however, there were many small leaf lesions and little stem blight. These results differ from those reported earlier (18), in which *F. roseum* caused stem lesions on red clover, but, apparently, not leaf spots. There were no clear-cut differences in the susceptibility of *Lotus* spp., although plants of 1 Danish line and those of 1 line of the Empire variety of *L. corniculatus* were much less severely affected than were all other lines tested.

Because *F. roseum* and other species of *Fusarium* are known to cause root rots in forage legumes (8, 11, 12, 13, 14, 19), an experiment was performed to test the pathogenicity of the red and brown strains of *F. roseum* to roots of *Lotus* spp. Steam-sterilized soil was infested by adding fragmented mycelium and spores of the fungus at the rate of 1 Petri-dish culture per 110 in.^{3} of soil. One- to 3-week-old seedlings of *L. corniculatus* var. *japonicus*, *L. corniculatus* var. Viking, *L. arenarius*, *L. divaricatus*, and *L. maroccanus* were transplanted to infested soil and to uninfested steam-sterilized soil. Eight plants of each species were transplanted to soil that had been infested with either the red or the brown strain of the fungus, and 1 plant of each species was transplanted into uninfested soil. Foliage symptoms were observed for 19 days after

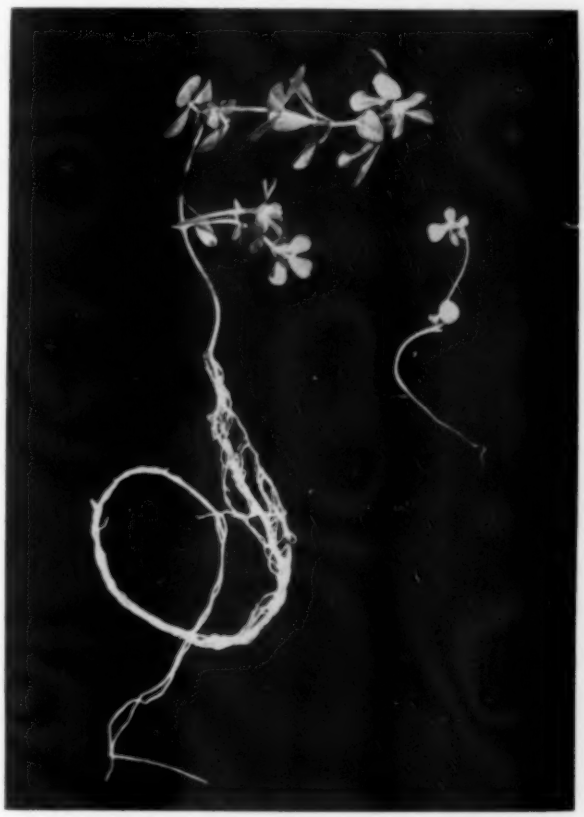

Fig. 2. Plants of *Lotus maroccanus*. Right, plant that had been transplanted 19 days earlier into soil infested with *Fusarium roseum*; left, plant that had been transplanted into uninfested soil 19 days earlier. All seedlings were the same age and size when transplanted.

[7] Wetting agent (sodium monolaurate) manufactured by the Atlas Powder Co., Chemicals Dept., Wilmington, Del.

transplanting, and root symptoms of remaining plants were then observed. No infection occurred on plants of *L. corniculatus* var. *japonicus* or *L. corniculatus* var. Viking, but roots of the other species tested were severely diseased (Fig. 2). Most of the susceptible plants were so severely affected that seedlings died within 10 days after having been transplanted into infested soil. No disease developed in plants grown in uninfested soil.

These results are considered to be proof that *F. roseum* is the primary causal agent of the blight of birdsfoot trefoil described here. Although *F. roseum* attacked the leaves and stems of alfalfa, red clover, and *Lotus* spp., and caused root rot of 3 species of *Lotus*, the strains isolated from blighted stems and leaves apparently are not important contributors to root rots of *L. corniculatus*.

SOME FACTORS THAT INFLUENCE INITIATION OF DISEASE.—*Preinoculation wounding.*—*Fusarium roseum* is known to gain ingress into plants through wounds (9). Although, in earlier experiments, the fungus was capable of initiating blight of birdsfoot trefoil in the absence of visible wounds, it was thought wounding plants before inoculation might facilitate ingress. In 1 experiment 6 plants were wounded by many needle punctures on apical portions of stems and leaves. These and 6 comparable but unwounded plants were inoculated with a suspension of fragmented mycelium and spores of *F. roseum*, and kept 2 days at 82°F and 100% RH. Plants were kept for 8 more days in a greenhouse at 66–78°F. Two uninoculated control plants were included in this test. Disease severity was rated 10 days after inoculation—from 0 for no disease to 10 for plant death from the disease. In this experiment, disease was moderate to severe on all inoculated plants. Average ratings for wounded and unwounded plants were 6.2 and 4.7, respectively; the difference was statistically significant at the 1% level.

Preinoculation wounding of test plants probably increased the number of infection loci, giving rise to more severe disease than in unwounded plants. Wounding was certainly not necessary for infection, however, and was not used in other experiments.

Kinds of inoculum.—Because the *F. roseum* isolated from birdsfoot trefoil sporulated profusely in culture, it seemed desirable to compare the infectivity of a spore suspension with that of a mixture of spores and fragmented mycelium. Spore suspensions were made by adding about 25 ml of sterilized distilled water to each of 6 Petri-dish cultures of the red strain of the fungus, and gently scraping the surface of the colony with a spatula. Spore suspensions were strained through cheesecloth. No fragments of mycelium could be observed in these preparations; spore concentration was such that about 13 macroconidia were observed in the 470× field of the microscope. Fragmented cultures were prepared as described earlier. Microscopic examination of this inoculum indicated that it contained about the same concentration of macroconidia as did the spore-suspension inoculum. Fourteen birdsfoot trefoil plants were inoculated with each inoculum, and incubated as described in the preceding section. Disease severity data were recorded 4, 8, and 10 days after inoculation.

Plants inoculated with a mixture of spores and mycelium were always more severely diseased than those inoculated with a spore suspension. Because spore concentrations were about the same in both inocula, fragments of mycelium probably were infective. A mixture of spores and fragmented mycelium was used in all other experiments reported herein.

Temperature.—Fusarium blight of birdsfoot trefoil was most severe in the Ithaca area during warm, wet periods in the summer. In 1957, for example, mean maximum and minimum temperatures were 79°F and 56°F for the 30 days preceding the outbreak of the disease.[8] Further, rain had fallen on 17 of those days. During the 8-day period ending 10 days before the disease was first observed, the average maximum temperature was 82°F, and rain had fallen on 7 of those days. The minimum temperature during that time, never lower than 57°F, averaged about 62°F. As reported above, disease developed more rapidly and became more serious in plants kept for 2 days after inoculation at 80–84°F than in plants kept at 70–78°F. In a separate experiment, 6 plants were inoculated as before and kept 2 days in a moist chamber at 90°F, and then in a greenhouse at 66–78°F. Only 2 of these plants became diseased, and symptoms were a few small necrotic spots on several leaves of each plant. *F. roseum* was isolated from these spots. It appears that 80–84°F favors Fusarium blight of birdsfoot trefoil more than does 70–78°F or 90°F.

The influence of temperature on the growth of the red and brown strains of *F. roseum* in culture was determined in 2 experiments. In one experiment, 30 ml of potato-dextrose broth[9] in each 125-ml Erlenmeyer flask used was infested with a 3-mm-diameter disk of mycelium, and PDA taken from the margin of a colony. Three flasks infested with each of the 2 strains of the fungus were kept 9 days at each temperature. Temperatures ranged from 37.4°F (3°C) to 92.3°F (33.5°C), at intervals of about 5.4°F (3°C). The amount of growth was determined by dry-weight measurements (15). In a second experiment, procedures were the same except that solidified PDA in Petri dishes was infested, and growth of the fungus was determined after 5 days by measuring the diameters of colonies as well as measuring dry weight. In these experiments, growth occurred at 42.8°F (6°C), but not at 37.4°F (3°C). On the liquid medium, growth was optimum at 77.2°F (24°C); on solidified PDA, however, growth was optimum at 82.6°F (27°C), whether determined by dry weight or by linear growth. Growth was about as great at 92.3°F (33.5°C) as at about 68°F (20°C). At temperatures above 65°F (18°C) the dry weight of the brown strain increased more rapidly than did that of the red strain, un-

[8] Climatological data for Ithaca, N. Y., 1958. U. S. Weather Bureau, Dept. of Commerce.

[9] Supernatant from 200 g cooked potatoes, 20 g dextrose, and distilled water to make a total volume of 1 liter.

doubtedly because of the abundant aerial mycelium produced by the brown strain. Rates of increases in diameter of colonies, however, were about the same, regardless of strain.

The optimum temperature for growth of *F. roseum* in culture is near the temperature that is most favorable for initiation of infection, but the fungus grows well in culture at 92.3°F, a temperature at which little or no disease develops. Temperature probably influences initiation of disease through its effects on susceptible plants and the pathogen or through its effects on their interactions, and not so much through its effects on growth of the pathogen alone.

Moisture.—Fusarium blight on field-grown plants was most severe during wet weather, indicating that moisture in the infection court might be essential to the initiation of infection. An experiment was performed to determine the lowest RH at which infection would take place.

Plants inoculated with suspensions of fragmented mycelium and spores were kept 2 days at 82°F—25 plants at 97–99% RH and 25 at 90% RH. At the higher humidity, moisture resulting from the inoculation evaporated completely from leaves in 10 hours; at the lower humidity inoculated foliage remained wet for 3 hours, but was dry in 6 hours. Two days after inoculation, all plants were moved to a greenhouse at 66–78°F.

No symptoms developed on plants incubated at 90% RH, nor could any fungus be reisolated from inoculated tissues. Small, discrete lesions developed on inoculated leaves of plants incubated at 97–99% RH; *F. roseum* was reisolated from several of these lesions. These results indicate that a wet period greater than 3–6 hours following inoculation is essential to infection. The fact that infection was only light on foliage kept wet for less than 10 hours indicates that disease will probably not be severe unless there is moisture in the infection court for more than 10 hours following inoculation.

DISCUSSION.—A *Fusarium* sp. has been reported to cause a "wilt (?)" of birdsfoot trefoil in New York (20). The strains of *F. roseum* isolated from blighted stems and leaves of birdsfoot trefoil certainly do not cause a vascular wilt, although they are capable of rotting the roots of *Lotus* spp. other than *L. corniculatus*. No published details of Fusarium "wilt" of birdsfoot trefoil have been found. This disease was reported to the Plant Disease Survey by M. F. Barrus[10] in August of 1942. The weather data for July 17-24, 1942 from Sullivan County, the area in which the disease was observed, indicate that conditions were similar to those that occurred in Ithaca during the summer of 1957. This suggests that the "wilt" reported earlier might have been identical to the stem blight reported here. Available data are insufficient, however, for drawing any conclusions on this point.

Because Fusarium blight of birdsfoot trefoil is favored by warm wet weather, it might be expected to be prevalent in the southern United States. The most likely explanation why Fusarium blight has not been reported from areas of climate warmer than New York is that *Rhizoctonia solani* is so prevalent in such areas that the disease it causes would be more noticeable than Fusarium blight.

From observational data taken in the field and from experimental data, it is clear that Fusarium blight of birdsfoot trefoil will be most severe during warm weather (80–84°F) and wet periods sufficiently long to account for moist infection courts for several hours. The lack of reports indicates that the disease has occurred rarely, if at all, in New York in the past, and that it probably will not often develop in epiphytotic proportions. Special environmental conditions necessary for development of the disease occur from time to time in the field in New York, but temperatures high enough for optimum disease development are not often long enough or accompanied by wet periods sufficiently long to account for extensive development of the disease.

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[10] Correspondence with J. A. Stevenson, Principal Mycologist in Charge of the U. S. D. A. Mycology and Plant Disease Reporting Section. The note on the report read: "Specimens sent by County Agent who said that the plants along the edge of fields wilted badly but later reported that, after cutting, the new growth did not appear to be affected. An undetermined species of Fusarium was isolated from mycelium found growing on outside of vines."

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RESISTANCE OF ONION TO DOWNY MILDEW[1]

S. Z. Berry[2]

SUMMARY

Resistance to *Peronospora destructor* in the onion strain 13-53 was compared with that in susceptible 2215 and 10 other susceptible strains and varieties. The resistance of the foliage and the immunity of the seed-stalks of 13-53 were found to break down when high humidity was maintained for periods longer than required for the initiation of infection in susceptible plants. The establishment of infection was affected by moisture, sporangiospore concentration, stage of development of the host attacked, and removal of the bloom from the plant surfaces. These factors, studied *in situ*, affected spore germination, germ-tube growth, and penetration, on detached leaves and seedstalks; where this growth was reduced, infection was also decreased. No association was found between the resistance and structure of the bloom and epidermis, stomate number and size, water deposit on the plants, and the contact angle. Once infection was initiated in 13-53, mycelial growth was less than in any of the other 11 onion strains studied. This inhibition was greater in the seedstalks than in the foliage of 13-53. Other than a slower rate of growth of the pathogen in the tissue of leaves and seedstalks of 13-53 vs. 2215, histologic changes of the host and development of the pathogen during infection could not be related to resistance. The resistance was resolved into 2 interacting factors. The first was the external resistance level expressed by the spore load necessary for infection, which could be manipulated by varying the inoculation conditions. The second was a factor that inhibited the pathogen following the establishment of infection.

INTRODUCTION.—An inbred line, Brigham Yellow Globe 2215, used as the male parent in 10 of 12 new U. S. D. A. hybrid onion introductions, was recently found to be susceptible to downy mildew (*Peronospora destructor* (Berk.) Casp.). As part of a program for incorporating the resistance of Italian Red 13-53 into susceptible Brigham Yellow Globe 2215, these studies were undertaken to secure a better understanding of the nature of this resistance. The former (resistant) strain is herein referred to as 13-53, and the latter (susceptible) strain as 2215.

Davis and Jones[3] developed the resistant variety Calred, which has moderate foliage resistance and high seedstalk resistance. The source of this resistance was 13-53, a selection by Dr. H. A. Jones, the leaves of which were only slightly injured under the most severe epiphytotic conditions. The seedstalks of this strain under the most extreme conditions of field infection were apparently immune.

MATERIALS AND METHODS.—Uniform inoculum for these experiments was secured by utilizing sporangiospores of *P. destructor* produced 18 days after inoculation, this period having been found long enough for sporulation when humidity is sufficiently high. Because this pathogen will sporulate only after exposure to light, the infected plants were placed in the moisture chamber in the evening at 5:00 P.M. and the pathogen allowed to sporulate. Spores were collected from the plants in the moisture chamber the day before being used as inoculum. Because necrosis of the host gradually develops after sporulation, the pathogen was transferred to healthy plants about every 3 weeks. The original inoculum of *P. destructor* used in this study was obtained from Dr. C. E. Yarwood, University of California, Berkeley.

To study the effect of incubation periods of different duration on resultant disease intensity, in each treat-

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From a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, University of California.
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[3] "Memorandum to Seedsmen: Release of Calred Onion," March 3, 1947. California Agricultural Experiment Station and the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. D. A.

ment 5 plants each of 2215 and 13-53 were used for each indicated exposure period. The plants were all inoculated in 40-gal. steel drums in a 10°C constant-temperature room. After the respective incubation periods the plants were removed from the moisture chamber and dried by an electric fan. This method of producing different incubation periods was based on techniques developed by Cook (2) and Yarwood (9).

Several factors were considered in arriving at a disease rating for infected foliage: The extent and intensity of symptoms and sporulation were graded individually on a scale of 0–4, from no disease to severe disease. The percentage of leaves infected was also rated on this scale, from no infection to infection of all leaves. An average of the three estimates—extent, intensity, and percentage of leaves infected—for each of the five plants in each incubation treatment was the disease rating. Seedstalk infection was rated on the basis of percentage of seedstalks infected and intensity of disease on each seedstalk.

For the study of the incubation stage on detached leaves and seedstalks, the respective tissues were cut into pieces 5 cm long by 2 cm wide; these were laid on moist paper towels in the bottom of a 4-gal. cylindrical jar. A water suspension of sporangiospores was then atomized onto the plant pieces. When dry inoculum was used, the sporangiospores were dusted on directly from plants on which sporulation had taken place. After inoculation the jar was covered, first with moist cheesecloth and then with a metal lid over this. Inoculation and incubation were carried out in a 10°C constant-temperature room.

Celloidin strippings were used for the *in situ* study of per cent germination, germ-tube growth, and stomatal penetration of resistant and susceptible plants. The celloidin method was similar to that described by Delp (3). Because of the irregular growth of many of the germ tubes, a camera lucida was used to trace their outlines, and figures so drawn were measured with a linear integrator.

For quantitative determination of the difference in growth of the pathogen in plants, specific areas on the foliage and seedstalks were inoculated by employing pieces of potato-dextrose agar about 0.16 in.^{2} seeded with sporangiospores. The agar was seeded with sporangiospores by spreading a concentrated suspension of the spores over the solidified agar in a Petri dish. In these tests 13-53 was compared to 2215 as well as 10 other onion strains—13-53 × 2215, Calred, Australian Brown, Lord Howe Island, California Red 21, Early Grano, Sweet Spanish, Stockton G 36, Australian Flat White, and Crystal Grano.

In addition to measurement of relative fungus growth (based on the extent of sporulation), in certain of the studies the extent of the internal vegetative mycelial growth was also determined. To accomplish this, entire leaves in which infection had developed were cleared in hot lactophenol and then stained for 10 minutes in hot 0.1% cotton blue in lactophenol. The mycelium and haustoria, which stained darker blue than the surrounding host tissue, could then be distinguished, and the extremities of the mycelial growth up and down the leaf determined.

In the study of the plant surfaces of resistant and susceptible plant material several different techniques were used in attempting to correlate resistance with surface characteristics. Material was embedded in paraffin as well as in water-soluble wax. Comparative studies of fresh material were also made. The technique that gave the most nearly normal picture of the plant surface was one using epidermal strippings. Pieces of epidermal tissue of 0.5 cm.^{2} were carefully stripped off and floated on water on slides, and a cover slip was placed over each without applying pressure.

For histologic and cytologic study, material was prepared by fixation in formalin–acetic-acid–alcohol, and sections were cut, free-hand as well as with the freezing microtome. For permanent mounts, material was embedded in paraffin, sectioned at a thickness of 10 μ, and stained with Conant's (4) quadruple stain.

RESISTANCE IN THE FIELD.—Since natural infection could not be depended on at Davis, California, the

TABLE 1.—*Relative severity of downy mildew infection on onion foliage and seedstalks*

Strain	Davis plots [a]						Milpitas plot [b]	
	1953-54 infection		1955-56 infection					
	Leaves		Leaves		Seedstalks	Disease	Seedstalks	Disease
	(number)	(%)	(number)	(%)	(number)	rating [c]	(number)	rating [c]
13-53	533	8	432	11	212	0	173	0
2215	242	45	140	53	80	3	68	4
13-53 × 2215	311	48	144	47	144	1	133	1
Calred	283	45	158	55	152	1	137	1
Australian Brown	265	54	185	64	102	4	109	4
Lord Howe Island	269	54	132	53	104	3	93	2
California Red 21	274	49	196	72	113	3	106	3
Early Grano	261	49	182	66	94	2	83	1
Sweet Spanish	276	49	188	71	102	3	100	3
Stockton G36	271	46	207	75	123	3	104	4
Australian Flat White	230	59	208	83	80	3	76	3
Crystal Grano	263	49	154	59	104	2	112	2

[a] Plots were inoculated by spraying with water suspension of sporangiospores.
[b] Natural infection.
[c] On a scale of 0–4, from no disease to severe disease.

plants in the field plots were sprayed with a water suspension of sporangiospores during weather conducive to the establishment of an epiphytotic. However, one test—at Milpitas, California—was based on natural infection. Studies in the field covered foliage resistance, seedstalk resistance, and the relative resistance of plants of different ages.

In 1953, 1954, 1955, and 1956, strain 13-53 was compared to 11 other strains considered susceptible. A randomized-block statistical design was used. In all tests the high field resistance of 13-53 foliage was observed (Table 1). The disease rating of 13-53 was distinctly different from that of any of the other strains, while there were no consistent differences in susceptibility evident among the other strains. As indicated, 13-53 foliage did show some infection, and the areas that occasionally became infected were the same as those on susceptible plants that usually showed severest infection. These areas were the older portions of the plant (the older leaves and the tips of the younger leaves). The range of seedstalk resistance was greater than observed in foliage since 13-53 seedstalks expressed complete field immunity (Table 1).

In view of the differences in resistance associated with the growth of the plant in the field, a study was made of the resistance of plants of different ages. The statistical design of this experiment was a split plot, with the different planting-time treatments as subplots and with a randomized-block layout. This enabled a comparison of infection on plants of different ages growing adjacent to each other. During a 2-year period, no consistent differences were observed in the resistance of plants of different ages. A total of 1456 plants, 2, 4, 6, and 8 months of age at inoculation, were studied.

RESISTANCE OF FOLIAGE UNDER SEVERAL CONDITIONS OF CONTROLLED INCUBATION.—Preliminary studies showed that factors involved in the expression of foliage resistance were length of the incubation period, sporangiospore concentration, and bloom on the leaf surface. Infection was consistently initiated for all the treatments only after at least a 2-hour incubation period. After an incubation period of 3 hours the disease rating for all treatments accelerated. Fig. 1-A illustrates this increase in disease intensity on 3 ½-month-old plants of 13-53 and 2215. Selection 2215 was consistently more susceptible. This was also true of foliage of 2215 and 13-53 that had been wiped, and the disease ratings increased with the longer incubation periods.

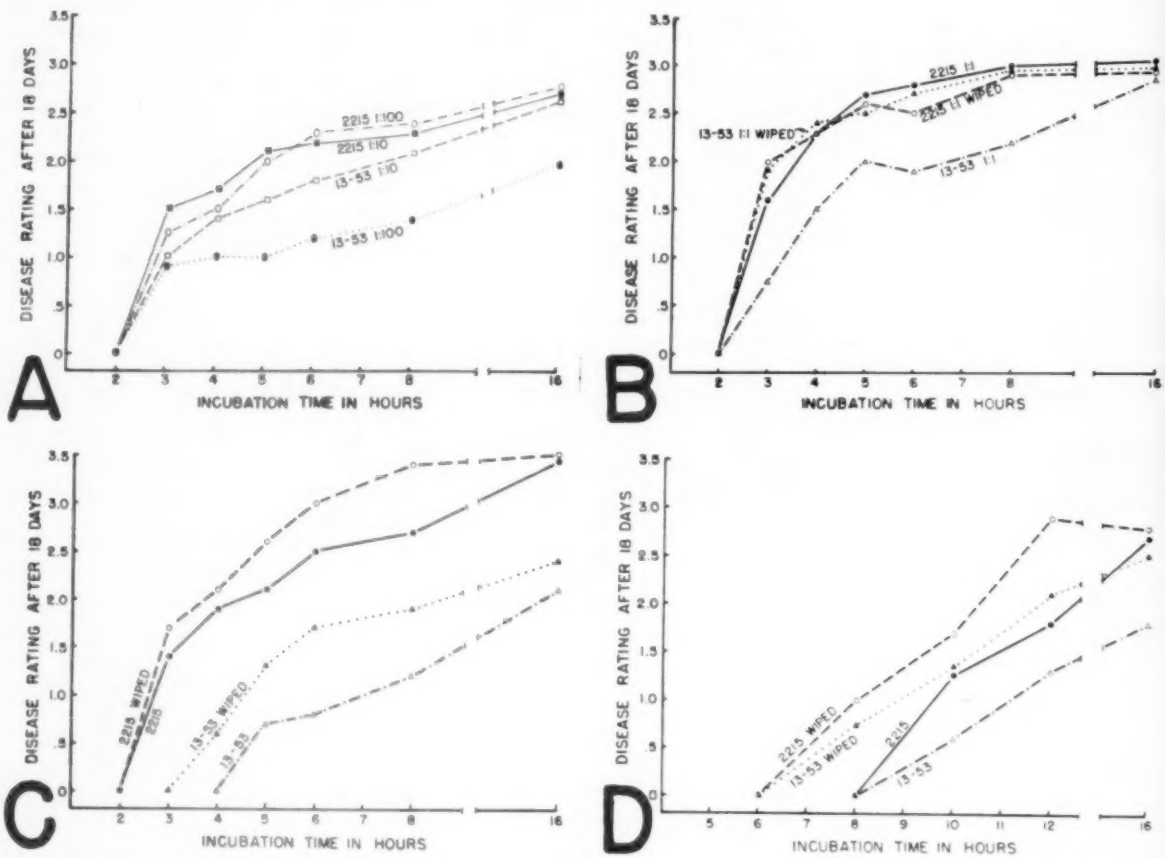

Fig. 1. A, B) The effect on subsequent infection of length of the incubation period, sporangiospore concentration, and wiping the leaf surface. Disease rating (0–4) is the average of 3 estimates: extent and intensity of infection, and percentage of leaves infected. **C)** Infection on seedstalks of 13-53 and 2215. **D)** Infection when inoculum is dusted on foliage directly from leaves on which the pathogen sporulated.

Fig. 1-A, B illustrate the modifying effect of different spore concentrations. In this study as well as in subsequent tests, an attempt was made to make a spore concentration as uniform as possible by using an original spore concentration of 25 spores per low power (10×10) microscope field. The original spore concentration was then diluted to provide a 1:1, 1:10, and 1:100 dilution series. A volume of 25 cc of inoculum was sprayed on each group of 5 plants used for each incubation time. The plants were incubated 10 hours at 10°C in separate moisture chambers. The differences were more distinct where the spores were diluted 1:10 and 1:100, and for the incubation periods of shorter duration. The plants of 13-53 and 2215 whose leaves were wiped with cotton before inoculation showed greater disease intensity than the respective treatments not wiped (Fig. 1-B,C,D).

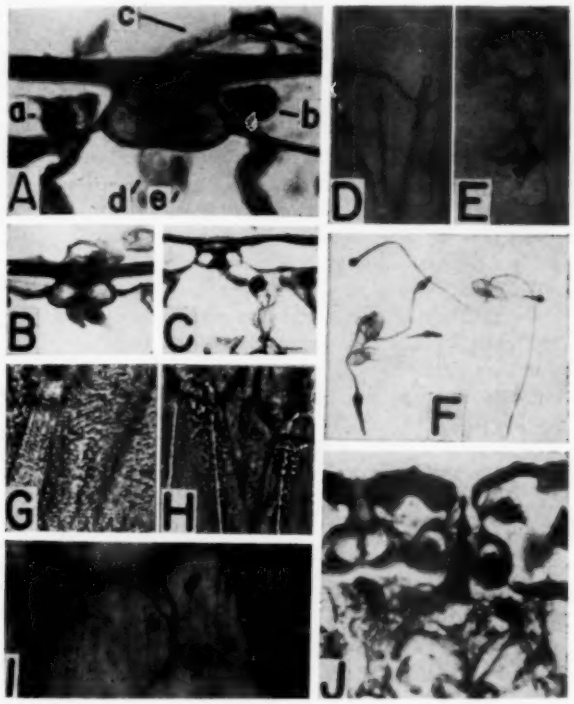

Fig. 2. A, B, C) Photomicrographs of radial sections of leaves penetrated by *P. destructor*. **A)** 2215; 48 hours after inoculation. Nuclear traumatotactic effect of 2 nuclei "a" and "b," remains of germ tubes and appressorium "c," substomatal vesicle "d," and infection hypha "e" (×540). **B)** 2215; 24 hours after inoculation. Divided vesicle formed in substomatal chamber (×400). **C)** 13-53; 24 hours after inoculation. Infection hypha and the palisade cell nucleus shown in close association (×260). **D, E)** Photomicrographs of *P. destructor* mycelium growing out of infected 13-53 tissue into the cavity of the leaf. (×240). **F)** Photomicrograph of germinating *P. destructor* sporangiospores, the germ tubes of which have formed appressoria (×240). **G, H)** Photomicrographs of the epidermal strippings of leaves of 2215 (×800). **G)** Leaf not wiped before stripping made. **H)** Leaf wiped before stripping made. **I)** Photomicrograph of a piece of 13-53 cleared leaf from the edge of a downy mildew lesion (×85). **J)** Photomicrograph of a section of an infected seedstalk of 13-53 on which sporulation has occurred (×340).

FACTORS AFFECTING SEEDSTALK RESISTANCE.—In view of the lack of complete immunity of the foliage of 13-53 in the field, the infection produced by artificial inoculation is not unusual. However, even under the most severe field epiphytotics, no infection has ever been observed on 13-53 seedstalks, which consequently have been termed immune (5). Yet, when seedstalks of 13-53, 2215, and 10 other strains were transplanted from the field, inoculated, and incubated for 10 hours in the moisture chamber, the seedstalks of 13-53 as well as those of the other strains became infected. Thus, the immunity of 13-53 was broken down (Fig. 2-J).

In view of the relative differences in infection on 13-53 and 2215 foliage with the different incubation periods, a parallel study was carried out on the seedstalks of 13-53 and 2215. There were 15 seedstalks in each treatment. Infection on unwiped as well as wiped seedstalks of 2215 was found to occur after an incubation period of 3 hours. Wiped 13-53 seedstalks became infected after an incubation period of 4 hours, whereas infection on unwiped seedstalks did not occur until 5 hours of incubation. In all treatments the disease rating increased with the longer incubation periods. Although this increase in disease intensity occurred, the difference in initiation of infection remained distinct between the different treatments (Fig. 1-C). This result is in contrast to the behavior of inoculated foliage, on which, after incubation for 8 and 16 hours, disease reached almost equally severe proportions regardless of treatment.

Field study of the breakdown of seedstalk immunity.—For effectiveness, the field study of factors affecting seedstalk resistance had to be carried out under conditions of high humidity. Such studies were made at night during wet weather in the springs of 1955 and 1956, when the necessary high humidity and moisture could be depended on.

A water suspension of sporangiospores was sprayed onto the foliage and the seedstalks in the evening during heavy mist or while it was raining. Steel drums were inverted over the inoculated plants to prevent the spores from being washed off. The drums were supported above the soil level by wooden blocks, leaving the openings in the drums otherwise unobstructed. In one such test, plants in the field that had produced seedstalks and still had heavy foliage were selected. Eight groups, each consisting of 2 plants of 13-53 and 2 of 2215, were sheltered from the rain by steel drums supported over the plants. The plants were then inoculated by atomizing the inoculum upward through the opening in the bottom of the drum. At 8:00 P.M., and at 1-hour intervals thereafter, 2 plants each of 13-53 and 2215 were dug up and transplanted to #10 cans in a greenhouse. As each group was brought into the greenhouse it was dried with an electric fan. After an infection period of 18 days, the infected plants were placed in a moisture chamber to induce the pathogen to sporulate.

Results showed that 3 hours was sufficient for the initiation of infection on both foliage and seedstalks

of 2215, whereas 13-53 remained completely free of infection. After the 4-hour incubation treatment there was an increased amount of infection on 2215, and small lesions appeared on 13-53 foliage. In the subsequent treatments, infection became more severe on both the seedstalks and foliage of 2215 and the foliage of 13-53. However, it was not until the 9-hour incubation treatment that infection was initiated on the 13-53 seedstalks, although in preliminary tests of this type in the field the incubation time for the initiation of infection on 13-53 seedstalks varied from 6 hours to 11 hours.

Thus, by maintaining moisture on plant surfaces resistant under field conditions, the sequence of breakdown in resistance, observed for plants inoculated and maintained in the moisture chambers for extended incubation periods, was duplicated in the field, and the immunity of 13-53 seedstalks was broken down.

CHARACTERISTICS OF PLANT SURFACES IN RELATION TO RESISTANCE.—Studies of the plant surfaces were made to determine whether any physical characteristic could be related to resistance. Alteration of the plant surface, as by wiping, and the variation of incubation conditions, particularly in relation to moisture deposit, indicated an external resistance factor.

An intensive study of the plant surfaces of 13-53 and 11 other onion strains revealed no differences that could be correlated with resistance. The layer of wax covering the plant surfaces of all the onion strains appeared smooth on macroscopic observation, but, when studied microscopically, was found to be made up of a layer of uniformly distributed projections of wax. These projections give the plant surfaces a granular appearance under the microscope (Fig. 2-G). By microscopic study it was found that wiping the plant surfaces with dry cotton removes the wax and destroys the granular-like pattern of the waxy covering (Fig. 2-H). No qualitative differences could be discerned, such as barrier structures on the epidermis or in the stomatal aperture, that might be associated with resistance. That the stomates of resistant plants do not in some way present a barrier was demonstrated in a subsequent study of the pathogen *in situ*, in which it was found that the number of penetrations was about the same on detached 13-53 leaves as on 2215 leaves (Table 4).

Epidermal strippings were used in making quantitative measurements of stomate density and size. Such strippings were made on 12 different varieties of plants 2, 4, and 6 months in age. They were taken from the distal, middle, and basal regions of the youngest, middle-aged, and oldest leaves. Similar measurements were made on the seedstalks of these 12 varieties (Table 2).

On 2-month-old plants of all varieties tested, stomate density of the youngest leaf decreased from the leaf base to the tip. In older leaves, the tips had a higher stomate density than the bases. Stomate length followed a reverse trend, with length increasing toward the tips of the youngest leaves, but there was little consistent difference between stomate lengths on the leaf areas of older leaves. Similar trends were found on the different leaves and areas of the leaves of 4- and 6-month-old plants. The lengths of the stomates in the epidermis of the seedstalks were consistently less, and their density was greater, than that of any of the stomates studied on the leaf areas. Certain trends related to the age and development of the plant were

TABLE 2.—*Stomate density* [a] *and stomate aperture length* [b] *in epidermis of onion leaf and seedstalk* [c]

	Stomate numbers			Aperture length (μ)		
	Resistant	Susceptible		Resistant	Susceptible	
Plant part	13-53	2215	10 others [d]	13-53	2215	10 others [d]
November, 1955						
Youngest leaf:						
Distal	8.4	9.9	7.3	12.3	14.9	13.7
Middle	7.6	11.2	7.6	12.9	8.9	11.7
Basal	10.5	15.2	13.8	10.3	6.6	8.6
Middle-aged leaf:						
Distal	6.7	9.0	6.3	12.0	11.1	13.7
Middle	5.1	8.2	4.3	13.4	12.0	13.7
Basal	6.3	5.8	5.1	11.1	12.3	12.0
Oldest leaf:						
Distal	5.1	7.8	5.2	12.6	10.3	13.7
Middle	4.2	8.2	4.8	11.4	10.6	14.3
Basal	2.9	2.8	3.0	12.6	12.6	13.4
May, 1956						
Seedstalk:						
Distal	4.2	3.8	0.8	16.0	16.3	15.4
Middle	5.1	4.2	3.0	13.4	13.7	16.0
Basal	3.0	3.3	1.8	14.3	11.7	14.9

[a] Average of 20 counts and measurements each for 13-53 and 2215 per low-power field (area = 85,530 μ^2).

[b] Stomate aperture length in μ. Average of 10 counts and measurements for each of 10 susceptibles.

[c] All seed was planted on September 20, 1955, and plants were grown in the greenhouse. The seedstalks were grown in the field.

[d] Calred, 13-53 × 2215, Australian Brown, Lord Howe Island, California Red 21, Early Grano, Sweet Spanish, Stockton G36, Australian Flat White, Crystal Grano.

thus observed in stomate density and size, but there was no correlation with resistance.

Quantitative estimates of the adherence of water to onion plant surfaces.—Yarwood (10) reported that, when water was sprayed on onion plants grown in the greenhouse, less water adhered to the leaves of plants of intermediate susceptibility than to leaves of highly susceptible plants. However, in the field at Milpitas, he could not find any marked difference in the retention of rain water or dew by the leaves of several onion strains with known differences in resistance. The writer similarly observed in the greenhouse that there were differences in the relative amounts of water that adhered to varieties known to differ in resistance (Table 3). This was also observed in the field after rain, but there was no association between resistance and water adhesion.

Two further studies were made of water adherence by measuring the amount of water adhering to a plant following spraying and the relationship between water droplets and the plant surface as measured by the contact angle. A smaller deposit of water was found to adhere to 13-53 foliage than to the foliage of any of 11 susceptible strains. With seedstalks there

TABLE 3.—*Adherence of water to foliage and seedstalks of onions*

Strain	Deposit of water spray [a] (mg/cm² of surface)		Adherence rating for foliage in field [b]
	Leaf	Seedstalk	
13-53	3.0	1.4	1.6
Stockton G36	4.0	1.1	2.1
California Red 21	4.6	0.8	2.0
13-53 × 2215	4.7	1.4	1.5
Crystal Grano	4.7	1.4	2.7
Sweet Spanish	5.3	3.6	3.9
Australian Brown	5.4	1.8	3.9
Early Grano	5.5	3.0	3.6
Calred	5.5	1.5	1.4
2215	6.2	4.3	1.6
Australian Flat White	6.5	2.0	1.7
Lord Howe Island	7.0	3.8	3.5

[a] Measurements were taken on 10 plants of each of the 12 stains, from May 16 to 20, 1956.

[b] A rating of 4 indicates almost complete coverage of the foliage with water; 0 indicates no water accumulation. Readings taken April 6, 1956, in the morning following rain. In the plot were 10 replications of each strain, with readings taken on a total of 480 plants.

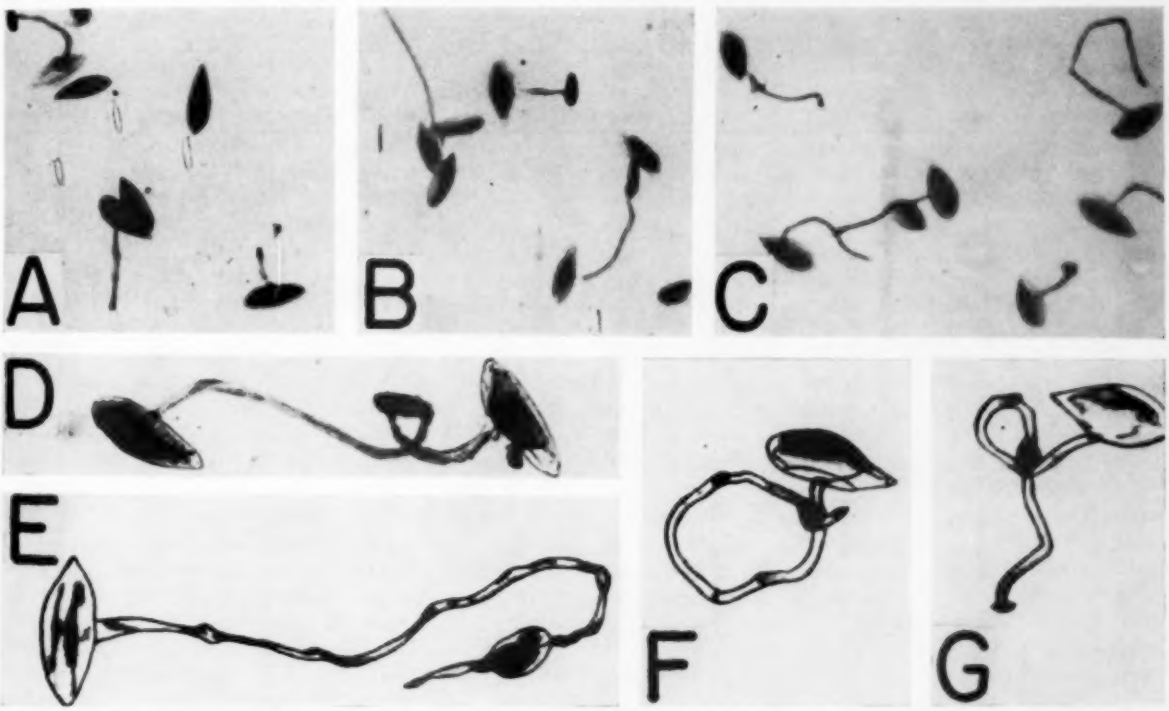

Fig. 3. *P. destructor* sporangiospore germination, growth and penetration as observed on celloidin strippings of the leaf, stained in cotton blue; **A–D)** photomicrographs, and **E–G)** line drawings. **A, B)** Development on the leaf surface of 13-53 (A) and 2215 (B) of *P. destructor* sporangiospores after incubation for 6 hours. **C)** Development on a 13-53 leaf 8 hours after inoculation by sporangiospores without free water. **D)** Penetration of a single stomate of 13-53 by 2 germ tubes (×885). **E)** Formation of an appressorium over a stomate of 2215 followed by a continued growth on the leaf away from the appressorium, 16 hours after inoculation with a water suspension of sporangiospores (×885). **F)** Appressorial formation by a growing germ tube that has contacted itself on a 13-53 leaf inoculated with dry inoculum and incubated 10 hours (×885). **G)** Same as F, but with continued growth of the germ tube after appressorium formation and ultimate penetration of a stomate upon formation of a second appressorium (×885).

was no relation between resistance and the adherence of atomized water. However, it was found that the average deposit on the leaves was 5.2 mg/cm^2, whereas that on the seedstalks was markedly less, 2.2 mg/cm^2. Study of the physical relationship between water and the plant surface by measurement of the contact angle showed that this characteristic could not be associated with resistance.

STUDIES OF THE PATHOGEN IN SITU DURING INCUBATION.—*P. destructor* was studied on wiped and unwiped plant surfaces of 13-53 and 2215 for different periods relative to percentage of germination, germ-tube growth, and the number of germ-tube penetrations of the host. In one phase of the study a water suspension of inoculum was atomized onto detached leaves, which were then incubated. In another phase, inoculum was dusted directly onto detached leaves from plants on which the pathogen sporulated, and the detached leaves were then incubated. The variability encountered in such studies was large. For example, the coefficient of variability of germ-tube length averaged 36% as determined in 20 measurements made on the foliage of each of strains 13-53 and 2215, 8 and 16 hours after inoculation. In this case, only germ-tubes that had not penetrated stomates were measured.

In these studies the only observed method of penetration was stomatal. Occasionally, on 13-53 and 2215 foliage, two germ tubes were observed to form appressoria over an individual stomate (Fig. 3-D). This phenomenon has not been previously noted for this pathogen. The formation of 2 vesicles in a substomatal chamber by the penetrating pathogen was also observed (Fig. 2-B). Another phenomenon not previously reported was the formation of an appressorium over a stomate, followed by continued growth of the germ tube on the leaf away from the appressorium (Fig. 3-E). The nuclear traumatotactic movement of nuclei in cells adjacent to penetrated stomates of onion foliage described by Yarwood (10) was observed on the foliage of both 13-53 and 2215 (Fig. 2-A). Epidermal strippings were examined under the microscope at hourly intervals after inoculation, and it was observed that very little nuclear displacement occurred before 8 hours had elapsed. This relatively long period indicates that resistance is not associated with this phenomenon, since infection can be initiated within 3–4 hours. Close contact between the nucleus of the

TABLE 4.—*Germination, germ-tube length, and penetrations (per low-power field) of Peronospora destructor on detached leaf pieces of 13-53 and 2215 at 10°C and 100% relative humidity*

Inoculum and incubation period (hours)	Treatment	13-53				2215			
			Tube length (μ)				Tube length (μ)		
		% germination [a]	Non-penetrating	Penetrating	No. penetrations	% germination [a]	Non-penetrating	Penetrating	No. penetrations
Water-suspended sporangiospores									
2	U [d]	0	0	0	0	0	0	0	0
2	W [e]	0	0	0	0	0	0	0	0
3	U	4	39	36	0-1	5	32	28	0-1
3	W	4	37	44	0-1	2	40	40	0-1
4	U	10	60	52	0-1	17	82	53	0-1
4	W	19	57	46	0-2	24	57	66	0-1
5	U	12	101	49	0-1	10	103	55	0-1
5	W	19	89	59	0-4	14	117	54	1-2
6	U	27	131	71	0-6	25	124	79	3-7
6	W	38	183	72	4-7	32	155	78	3-8
8	U	43	215	195	2-8	56	215	147	3-9
8	W	60	240	188	4-10	54	186	127	2-7
16	U	68	367	209	2-10	68	349	324	2-8
16	W	76	353	199	5-7	81	409	223	3-7
Dry sporangiospores									
4	U [d]	0	0	0	0	0	0	0	0
4	W [e]	0	0	0	0	0	0	0	0
5	U	2	34	28	0-1	7	40	41	0-1
5	W	4	43	37	0-1	3	50	34	0-1
6	U	16	68	51	0-1	14	78	69	0-1
6	W	23	83	76	0-1	21	116	63	0-1
7	U	21	60	55	0-3	25	64	56	0-3
7	W	23	73	57	0-4	34	125	79	0-3
8	U	34	104	60	2-4	31	98	92	2-4
8	W	38	157	123	1-4	37	164	209	2-6
10	U	38	182	90	1-6	56	244	147	0-6
10	W	52	267	145	2-4	49	223	119	1-9
16	U	73	374	196	4-8	76	534	164	5-9
16	W	64	393	291	3-7	72	472	217	4-8

[a] Average of counts made in 5 low-power fields.
[b] 20 measurements averaged for each value given.
[c] 10 measurements averaged for each value given.
[d] Unwiped leaf treatment.
[e] Wiped leaf treatment (wiped with cotton before inoculation).

first palisade cell attacked and the infection hypha was also observed (Fig. 2-C).

Development of sporangiospores when applied in water suspension.—In the first type of experiment, inoculum was atomized onto the detached leaves, and differences in percentage of germination, growth of germ tubes, and germ-tube penetrations were studied over a range of incubation periods of 2, 3, 4, 5, 6, 8, and 16 hours (Table 4). A consistently greater per cent of germination on wiped plant surfaces than on unwiped is to be noted, but this greater activity was not consistently reflected in germ-tube growth or in the number of penetrations. Germination, growth, and penetration were greater from 6-hour incubation than from 5-hour incubation. This increase was in accordance with the infection initiated at the different incubation periods. The low threshold of infection of this pathogen is indicated by the relatively heavy infection produced by shorter incubation periods, as indicated in the previous sections, yet during these shorter incubation periods the percentage of germination, the germ-tube growth, and the penetrations were low.

Development of sporangiospores when applied dry.—In the second type of experiment, dry detached leaves of 13-53 and 2215 were inoculated by dusting sporangiospores onto the plant surfaces and incubating at 10°C and 100% RH (relative humidity) for 2, 3, 4, 5, 6, 7, 8, 10, and 16 hours (Table 4). It was found that sporangiospore germination and germ-tube growth were not appreciable until a 5-hour period had elapsed. Similarly, infection was not initiated until a 5-hour period had elapsed. This period of 5 hours is contrasted with the 3 hours required for the intiation of infection when sporangiospores were atomized onto the plant surfaces. In these tests, when germination once started, the development on 13-53 and 2215 at the corresponding treatments increased progressively and showed accelerated development between the 7- and the 8-hour treatment. These results are analagous to the increase observed between the 5- and the 6-hour period in the case of inoculations with a water suspension of sporangiospores. Another significant observation was the relatively greater amount of germ-tube development on the wiped leaf surfaces. This condition was in accordance with the greater infection associated with wiped plant surfaces. The incubation periods necessary were found to be much longer when inoculum was free of moisture than when inoculum was sprayed onto the plants. In spite of the greater incubation time, the same disease intensity was observed as on comparable treatments with moisture present (Fig. 1-D). Wiped foliage became infected after an incubation period of 8 hours, whereas unwiped foliage was not infected until after 10 hours. There was consistently more infection on 2215 than on 13-53, with disease intensity increasing with length of incubation period. Since free water was absent at the time of inoculation, 100% RH alone might thus be considered sufficient for the breakdown in resistance. However, it was observed that, under these conditions, moisture was condensing on the foliage after 6 to 8 hours. Apparently, infection did not occur until water had condensed on the leaves.

TABLE 5.—*Measurement of mycelial growth on different onion strains of Peronospora destructor in leaf tissue compared with the length of the sporulating lesion* [a]

Strain	Mycelial growth			Sporulation (cm)		
	Up	Down	Total	Up	Down	Total
13-53	4.3	6.2	10.5	3.5	4.1	7.6
2215	5.7	10.3	16.1	5.1	6.7	11.8
13-53 × 2215	5.4	7.7	13.1	4.4	5.3	9.7
Calred	5.9	8.7	14.6	4.8	5.4	10.2
Lord Howe Island	5.6	11.7	17.3	4.0	6.5	10.5
Early Grano	6.2	8.6	14.8	5.6	5.7	11.3
California Red 21	7.2	9.4	16.6	6.0	7.6	13.6
Australian Brown	5.7	7.7	13.4	4.8	5.9	10.7
Sweet Spanish	9.1	8.3	17.4	6.2	5.2	11.4
Stockton G-36	5.3	8.7	14.0	5.0	6.3	11.3
Australian Flat White	7.3	9.3	16.6	5.5	7.3	12.8

[a] 18 days after inoculation of the midpoints of the leaves. Each figure for 13-53 represents an average of 20 measurements, and that for each of the other strains an average of 10 measurements.

Development of the pathogen on detached seedstalks.—Study of the pathogen *in situ* on detached seedstalks of 13-53 and 2215 was similar to the studies reported for detached leaves, but was approached only qualitatively. Although the seedstalk of 13-53 possessed a much higher level of resistance (field immunity) than the foliage, development of *P. destructor* on seedstalks of 13-53 and 2215 was similar to that on the foliage of these plants for the respective incubation periods in the case of both wet and dry inoculum.

GROWTH OF THE PATHOGEN IN RESISTANT AND SUSCEPTIBLE PLANTS.—Yarwood (10) noted that *P. destructor* grows downward in foliage more rapidly than it grows upward. In the present study this was found to be true, in the susceptible onion strains studied as well as in 13-53. Jones, Porter, and Leach (5) observed in the field that infection is usually confined to the tips of the leaves and that the pathogen grows very slowly toward the base. By inoculation with sporangiospore-seeded agar blocks at specific areas on the leaves of 13-53, it was found that *P. destructor* was capable of initiating infection in other parts of the leaf besides the tip.

The extent of sporulation was never as great as that of mycelial growth. However, sporulation measurement was a good indication of mycelial growth and was used in subsequent work since it was more practical than measuring internal mycelial growth (Fig. 2-I) to determine the relative extent of the pathogen's development (Table 5). By such means it was found that the relatively smaller extent of sporulation on 13-53 than on susceptible plants was related to a smaller extent of mycelial growth rather than an inhibition of sporulation.

Relative growth in leaves at different stages of development and in leaves of 2-, 4-, and 6-month-old plants.—The growth of *P. destructor* was determined for young, middle-aged, and old leaves. Inoculations were made on the middle of the leaves with sporangio-

spore-seeded agar blocks. It was found that the extent of sporulation on 13-53 was less than that on any of the other onion strains studied. However, there were no marked differences in the growth of the pathogen in leaves of different development. In one such test of 6-month-old plants, the youngest, middle-aged, and oldest leaves were inoculated on an average of 16 plants of each of 12 different strains. Sporulation was induced after 18 days, and the following average growth of the pathogen was found to have occurred, as indicated by extent of sporulation on the youngest, middle-aged, and oldest leaves, respectively: 3.7, 4.8 and 4.9 cm for 13-53; 10.2, 11.0, and 11.5 cm for 2215; and 9.9, 10.6 and 9.9 cm for the average of all the other 10 varieties.

Growth of *P. destructor* was studied in 2-, 4-, and 6-month-old plants of 13-53, 2215, and 10 other susceptible varieties. The plants used in each test were all seeded at the same time: on September 20, 1954, in the 1954–55 test, and September 20, 1955, in the 1955–56 test. Inoculations were made with sporangiospore-seeded agar blocks. No consistent relation could be found between plant age and extent of sporulation. However, less growth occurred in the 13-53 plants of all ages than in plants of any of the other onion strains tested.

Relative growth in seedstalks.—It was indicated that the field immunity of 13-53 seedstalks could be overcome by extended periods of high humidity after inoculation. Similarly, by inoculating with sporangiospore-seeded agar blocks, infection could be initiated in seedstalks and the growth of *P. destructor* could be studied. The seedstalks were grown in the field and transplanted into containers before being inoculated in the moisture chamber. An average of 24 seedstalks each of 13-53, 2215, and 10 other onion strains were inoculated on May 2, 1955, and a similar test was carried out on April 16, 1956. Sporulation was induced and measured 18 days after inoculation. The results were similar each year (Table 6). In both tests the average extent of sporulation was 2.9 cm on 13-53, 15.2 cm on 2215, and 15.3 cm on the other 10 strains. Berry and Davis (1) reported *P. destructor* oospores in both susceptible and resistant onion seedstalks.

TABLE 6.—*The length (cm) of sporulating lesions of Peronospora destructor on seedstalks of onion strains inoculated in 2 years*

Strain	May 2, 1955	April 16, 1956
13-53	4.0	1.8
Calred	12.2	9.5
Early Grano	13.5	10.3
Crystal Grano	18.5	11.2
13-53 × 2215	10.8	12.1
Lord Howe Island	12.1	13.7
Sweet Spanish	13.3	14.0
California Red 21	11.3	14.6
Australian Flat White	12.2	16.9
2215	13.0	17.3
Australian Brown	14.2	18.5
Stockton G 36	15.8	22.1

[a] Seedstalks grown in the field during the spring and transplanted into containers previous to inoculation in the moisture chamber. An average of 50 seedstalks of each variety were used. Inoculation was with sporangiospore-seeded agar blocks. Sporulation was measured 18 days after inoculation.

HISTOLOGY OF INFECTED RESISTANT AND SUSCEPTIBLE PLANTS.—Examination of infected tissue showed extensive intercellular mycelial growth and intracellular haustorial development of the pathogen in both 13-53 and 2215, and there was very little difference between the two as to the form and type of this development. However, as indicated previously by the measurement of mycelium and sporulation, the extent of development in both leaves and seedstalk was less in 13-53 than in 2215.

The traumatotactic movement of the nuclei in epidermal cells adjacent to penetrated stomates has been noted. Whetzel (8) observed the close association of the haustoria of *P. destructor* with the nuclei of infected tissue. Similarly, in this study, a close associa-

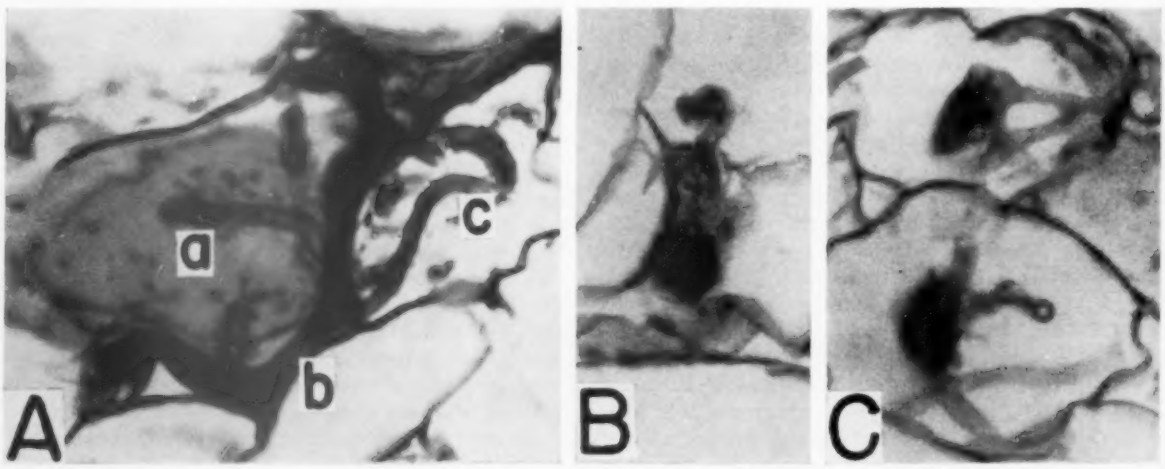

Fig. 4. Photomicrographs of cross sections of onion leaves infected with *P. destructor* (×600). **A)** 13-53, "a," intercellular mycelium; "b," haustorium surrounded by chloroplasts; "c," cell in which the cytoplasm has been completely disorganized. **B)** 2215, traumatotactic effect of nucleus—adjacent to the cell wall, intercellular mycelium, and haustorium. **C)** 13-53, haustoria and host nuclei in close association.

tion was found between the nuclei of the host and the haustoria of the pathogen in diseased internal tissue of both 13-53 and 2215 (Fig. 4). A traumatotactic relationship of the nuclei of host cells and the mycelium, as well as the haustoria of the pathogen, was also observed.

In both 13-53 and 2215 the pathogen was found to grow primarily through the chlorophyll-containing palisade and mesophyll tissues, and was also observed in the elongated cells below the mesophyll and adjacent to the vascular tissue. In this study, as far as the author is aware, the first observation was made of the growth of mycelium of *P. destructor* out of infected onion leaf tissue into the cavity of the leaf. In Fig. 2-D,E are illustrated 2 types of growth into the leaf cavity, straight branching and circling unbranched. No haustoria were observed on these growths.

The resistance of several hosts attacked by pathogens closely related to *P. destructor* has been attributed to an internal defense mechanism. Wang (7) studied the mechanism of resistance of cruciferous plants to *Peronospora parasitica* and found that immune and resistant hosts did not hinder entry of the pathogen. However, after entry, resistance was expressed by a necrosis of the host cells surrounding the invading hyphae, resulting in death of the fungus. Thomas, Jorgensen, and Wester (6) studied the inheritance of resistance to downy mildew, *Phytophthora phaseoli*, in lima bean, and indicated that the necrosis and lack of sporulation on the resistant lines were a form of resistance similar to the necrotic reaction of potatoes resistant to late blight.

In this study, the resistance in onion to downy mildew was inhibition of growth of the pathogen rather than rapid necrosis and complete halting of fungus growth. The pathogen was capable of continued development even after sporulating. Thus, when 24 infected leaves of 13-53 were inoculated at points by means of sporangiospore-seeded agar blocks, average sporulation per leaf was 1.6 cm after 11 days, and at 18 days had increased to an average of 7.6 cm. On 110 infected leaves of 11 susceptible onion strains, including 2215, sporulation per leaf averaged 2.9 cm at 11 days, and at 18 days had increased to 12.3 cm. However, it should be noted that, once sporulation did occur, necrosis developed rapidly, and that the pathogen died if conditions such as high temperature were not favorable for the pathogen to grow out of the infected areas into healthy areas of the plant. This latter phenomenon was observed to be similar in the 11 susceptible strains studied, as well as in 13-53.

THE EFFECT OF PLANT EXTRACTS ON THE GERMINATION OF SPORANGIOSPORES AND GROWTH OF GERM TUBES.—In general there was no marked difference between germ-tube growth on extracts of 13-53 and 2215 leaves. Similar results were secured with seedstalk extracts. There have been no reports in the literature of appressorium formation by *P. destructor* germ tubes on artificial culture media. However, appressorial formation was occasionally observed in this study on water agar to which very dilute extracts of 2215 and 13-53 had been added. The appressoria varied in form from triangular to circular (Fig. 2-F).

DISCUSSION.—In the field as well as in the greenhouse, the older leaves and the tips of the younger leaves are consistently more heavily infected. After exposure to extended periods of high humidity the resistant foliage and the immune seedstalks of 13-53 became infected. The degree of infection was decreased by the dilution of the inoculum, and increased if the wax bloom on the leaves was wiped before inoculation or if the sporangiospores were applied as a water suspension rather than dusted onto plants directly from leaves on which the pathogen had sporulated. No relation could be found between resistance and the adhesion of water to the different strains of onions, but it was consistently observed that water adhered in greater amounts to the older leaves and the tips of younger leaves—areas of the foliage that showed a proneness to infection. Frequent observation of the development of infection on bent-over leaves in the field with infection stemming from the horizontal bend indicated further the effect of moisture on the initiation of infection, since moisture accumulation and condensation are greater at such points.

Yarwood (9), in studying the relation of moisture to infection of various mildews and using conditions similar to those used in this study, found that in the inoculation of onion foliage with dry downy mildew sporangiospores, there was a condensation of moisture on leaves after an incubation period of 15 hours. Thus, he could not with certainty conclude that free moisture is unnecessary for the initiation of infection. As indicated, in this study incubation periods of 2–16 hours were used, and the appearance of visible condensation and the initiation of infection were found to be closely associated.

Study of the development of *P. destructor* on foliage and seedstalks *in situ* explained such phenomena as the increase in disease with longer moisture chamber incubation, the latter being attributable to the longer period allowed for penetrations. Similarly, the longer incubation period necessary for initiating infection when plants were inoculated with dry inoculum rather than moist inoculum was found to be due to the reduced rate of spore germination, and in turn delayed penetration by the pathogen. A higher threshold of infection for 13-53 than for 2215 could explain the different infection phenomena resulting from the different treatments, which in effect alter the spore load. Further evidence for the existence of such a factor was provided by tests in which infection was initiated in leaves and seedstalks, and as a result there was an inhibition of mycelial growth in 13-53 compared to that in any of the other 11 onion strains tested. This inhibition was consistent in plants of different age and leaves of different stages of development, and was also present to a still higher degree in seedstalks than in foliage of 13-53.

This study provides evidence that the apparent external and internal resistance of the onion to *P. destructor* is an expression of a single factor, which

exists at different levels depending on the resistance of the onion strain. The spore load modifies this factor, and if conditions are supplied that make the effective spore load sufficiently high, the resistance can be overcome; this is the case even in the seedstalk of 13-53, which possesses the highest level of onion downy mildew resistance known, being immune under natural epiphytotic conditions in the field.

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THE RELATION OF SAPWOOD MOISTURE CONTENT TO THE INCIDENCE OF MAPLE WILT CAUSED BY VERTICILLIUM ALBO-ATRUM[1]

Nestor E. Caroselli[2]

SUMMARY

Norway maple trees were inoculated with *Verticillium albo-atrum* and either permitted a natural water supply or provided with supplemental water applied directly into the sapwood. Average wilt and extent of sapwood discoloration was less in those given additional water. Severity of wilt symptoms and sapwood discoloration was less in trees having a greater sapwood moisture content.

Several investigators have demonstrated a relationship between the water content of wood and the activities of certain wood-inhabiting fungi. Experiments conducted by Dick (3), Muench (5), Snell (6, 7) and Snell, Howard, and Lamb (8) have revealed that the moisture content of wood is an important factor in determining the growth of decay fungi. Reference to a similar relationship in living trees has been made by Henriksen and Jørgensen (4).

The author (1, 2) has presented some evidence of the effect of sapwood moisture content in field-grown sugar maple trees upon the incidence of disease incited by *Verticillium albo-atrum* Reinke & Berth. The present investigation was conducted to compare the severity of disease, as expressed by wilt symptoms and sapwood discoloration, in inoculated Norway maple trees with the severity in trees similarly inoculated, but receiving additional water directly into the trunk throughout the season.

MATERIALS AND METHODS.—Twenty Norway maple trees varying in size from 3½ to 4 in. diameter breast height and having an average height of about 10 ft. were selected at random in a nursery plot. These were divided into 2 equal groups. One group procured water only by normal root absorption; the second was given supplementary water throughout the experiment. This constant supply of moisture was maintained by passing water from a 5-gal. reservoir can through rubber and glass tubing fitted with a bored cork inserted into holes made in the sapwood (Fig. 1). A hole of ⅜ in. diameter was bored radially into the tree to a depth of 1–1½ in., depending on the size of the tree. One tangential hole was drilled with a ⅛-in. bit on each side of the first boring (Fig. 1). Water was provided on the same day and prior to inoculation. Cans were refilled at about weekly intervals.

Sapwood samples were obtained periodically by a technique discussed in a previous paper (2). Dates on which samples were procured are noted in Table 1. Moisture content of the sapwood samples was expressed as percentage, and determined on fresh-weight and dry-weight bases,[3] but only the latter is reported in this paper. Test samples were weighed to obtain the fresh weight, dried 48 hours in a hot-air oven at 100°C, and reweighed.

To determine the maximum water-holding capacity of the sapwood, 1-cm³ blocks collected from 10 different uninoculated trees were aspirated *in vacuo* until they sank to the bottom of a water-filled container. Aspiration was then repeated at 2-hour intervals, for ½-hour periods, until no bubbles were emitted from

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[3] Percent moisture dry-weight basis $= \frac{\text{Fresh wt.} - \text{dry wt.}}{\text{Dry wt.}} \times 100$

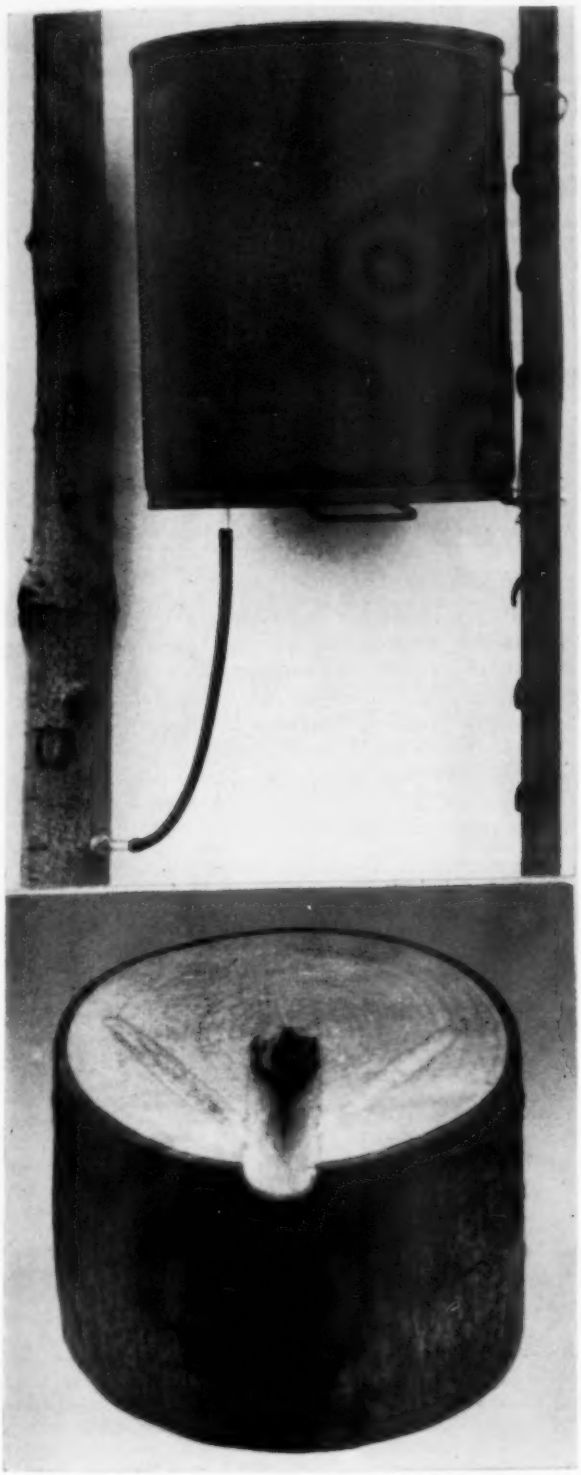

Fig. 1. Above) The apparatus used to maintain a constant supply of water to the sapwood. **Below)** A transverse section of a Norway maple, made through 1 radial and 2 tangential bore holes.

the wood. The average moisture content of the samples was determined to be 129.9% calculated on a dry weight basis.

On June 17, 1957, 5 trees of each group were inoculated by insertion of a 2% malt agar disk containing an actively growing colony of the fungus into vertical wounds made in the sapwood with a knife previously dipped into alcohol. Drying-out of the mycelium was prevented with a covering of moist absorbent cotton secured with a strip of adhesive tape. The five uninoculated trees of each group were similarly wounded, but only sterile agar disks were inserted. Subsequent wilt symptoms were estimated as per cent of the crown of infected trees.

On completion of the study, on February 10, 1958, all trees were cut down and studied to determine the extensiveness of sapwood discoloration. At this time, tissue platings were made on a 2% malt agar medium for re-isolation of the fungus.

DISCUSSION OF RESULTS.—The average per cent moisture of the inoculated and uninoculated trees, with and without an additional supply of water in each group, is recorded in Table 1 and expressed graphically in Fig. 2. It may be noted that the average moisture content of inoculated and uninoculated trees within each group are in reasonably close agreement, regardless of time of year. The sapwood moisture content of trees given an additional supply of water, however, was higher than that of the other group up to leaf fall (October 15, 1957). Then the differences decreased markedly, and moisture contents were practically equal at the "winter-green" condition (January 15, 1958). In most cases the winter-green condition was slightly less than the maximum water-holding capacity of the sapwood.

In this experiment there appeared to be a general tendency toward a decrease in wilt symptoms with an increase of the sapwood moisture content, and vice versa. Seven of the 10 inoculated trees exhibited various degrees of wilt by August 12, 1957. The average wilt of trees given an additional supply of water

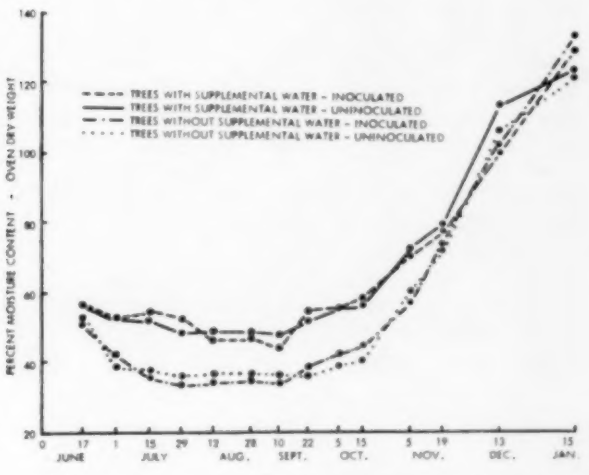
PERCENT MOISTURE CONTENT - OVEN DRY WEIGHT
140
120
100
80
60
40
20
TREES WITH SUPPLEMENTAL WATER - INOCULATED
TREES WITH SUPPLEMENTAL WATER - UNINOCULATED
TREES WITHOUT SUPPLEMENTAL WATER - INOCULATED
TREES WITHOUT SUPPLEMENTAL WATER - UNINOCULATED
0 17 1 15 29 12 26 10 22 5 15 5 19 13 15
JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN.

Fig. 2. Average sapwood moisture content of inoculated and uninoculated trees (dry-weight basis).

TABLE 1. *Moisture of the sapwood, degree of foliage wilt and extensiveness of sapwood discoloration of inoculated and uninoculated maple trees (dry-weight basis)*

Tree No.	Injected with water	Per cent water content of sapwood at indicated dates															Wilt %		Discoloration* (in.)	Reisolation
		6/17	7/1	7/15	7/29	8/12	8/28	9/10	9/15	9/22	10/5	10/15	11/5	11/19	12/13	1/15	7/1	8/12		
Inoculated																				
		50.3	44.5	51.6	46.8	43.9	46.6	48.8	51.3	55.3	58.7	61.5	66.7	79.6	98.4	128.2	0	0	1	+
4	+	57.7	54.2	53.1	51.5	47.3	50.7	54.6	59.3	62.0	53.2	57.9	70.1	84.2	109.6	134.9	0	5	1.5	+
5	+	58.6	59.3	56.6	54.8	51.3	43.1	43.8	41.6	53.1	56.9	59.5	63.7	59.7	105.4	131.8	0	15	2	+
6	+	59.2	48.4	55.4	58.0	52.8	55.2	34.8	38.7	57.0	53.4	61.5	65.9	62.1	102.4	120.8	10	40	3	+
7	+	56.6	59.9	55.1	54.9	43.1	40.9	42.5	69.4	45.0	57.3	56.8	85.8	105.5	89.3	130.8	0	0	1	+
Av.		56.5	53.3	54.4	53.2	47.7	47.3	44.9	52.1	54.5	55.9	59.4	70.4	78.2	101.0	129.3	2.0	12.0	1.7	
8	–	45.1	33.8	29.1	28.1	25.5	27.5	26.4	30.7	35.7	37.5	40.3	52.2	66.9	98.8	142.1	10	50	10.5	+
9	–	56.9	48.4	37.2	34.4	35.2	33.5	32.7	37.2	41.3	39.5	43.5	61.3	77.4	98.4	125.4	20	30	8	+
10	–	52.3	43.2	41.5	37.5	35.4	37.4	37.5	40.0	42.6	42.0	46.3	56.1	67.0	104.5	118.8	0	0	5.5	+
14	–	50.8	48.8	37.3	41.8	47.1	48.0	47.3	47.4	45.5	55.6	49.4	56.4	79.3	102.0	118.2	5	20	4	+
15	–	55.2	41.4	31.2	31.3	32.7	33.0	30.2	32.6	33.2	36.9	42.4	59.3	72.5	106.7	149.5	20	70	13	+
Av.		52.1	43.1	35.3	34.6	35.2	35.9	34.8	37.6	39.7	42.3	44.4	57.1	72.6	102.1	130.8	11.0	34.0	8.2	
Uninoculated																				
1	+	55.5	45.7	50.7	48.4	39.8	43.3	45.9	51.5	55.3	55.6	54.2	67.0	70.0	104.1	100.0	0	0	0	–
3	+	61.8	59.0	56.2	50.8	51.9	53.8	53.8	56.2	54.1	63.1	59.2	79.9	77.6	133.7	149.5	0	0	0	–
11	+	53.6	54.4	48.6	42.1	50.7	53.1	47.9	54.3	51.2	56.1	56.7	70.3	75.1	112.7	118.2	0	0	0	–
12	+	60.4	56.0	53.1	54.4	46.9	50.8	52.0	55.4	46.5	50.1	59.6	79.5	84.5	120.3	142.1	0	0	0	–
13	+	51.6	54.0	55.4	50.7	53.7	44.0	41.1	48.3	53.1	55.3	56.1	65.6	86.6	100.3	104.1	0	0	0	–
Av.		56.6	53.8	52.8	49.3	48.6	49.0	48.1	53.1	52.0	56.0	57.2	72.5	78.8	114.2	122.6	0	0	0	
16	–	54.2	43.2	42.1	38.1	27.0	32.0	29.2	31.1	33.4	36.3	41.1	65.5	72.7	114.2	123.6	0	0	0	–
17	–	47.0	34.4	37.3	36.8	38.8	42.1	37.0	41.4	40.5	43.0	51.8	68.4	89.3	121.3	128.2	0	0	0	–
18	–	49.6	32.1	30.8	35.1	37.4	39.0	37.4	32.1	33.5	40.1	37.3	64.7	80.0	106.6	104.1	0	0	0	–
19	–	57.9	43.3	40.2	33.9	39.3	35.6	34.6	36.4	33.9	38.7	36.8	53.1	64.8	92.0	128.9	0	0	0	–
20	–	54.4	42.7	41.3	36.4	37.4	32.8	35.7	38.8	39.5	38.6	35.1	49.5	60.7	100.3	120.3	0	0	0	–
Av.		52.6	39.1	38.3	36.1	35.9	36.3	35.4	36.0	36.2	39.3	40.4	60.3	73.5	106.9	121.0	0	0	0	

*The fungus was reisolated only from the inoculated trees.

was less, and the sapwood moisture greater, than that of similarly inoculated trees receiving none. The former displayed 2% and 12% wilt, and 53.3% and 47.7% sapwood moisture, for July 1, 1957 and August 12, 1957, respectively, whereas the latter exhibited 11% and 34% wilt, and 43.1% and 35.2% sapwood moisture for the same dates. During the period July 1 to August 12, 1957, the average sapwood moisture content of these two groups was 52.2% and 37.0%, respectively. The average sapwood moisture for the same groups during the period June 17 to October 15, 1957, was, respectively, 52.7% and 39.5%.

There was an indication that the extent of sapwood discoloration decreases with an increase of the sapwood water content. Considering the same period and moisture contents mentioned above for inoculated trees, with and without added water, the ranges in sapwood discoloration above the point of inoculation were 1–3 in. and 4–13 in., respectively (Table 1). The average measurable discoloration was 1.7 and 8.2 in.

Although most of the inoculated trees showed a correlation between the amount of sapwood water and the severity of wilt and extensiveness of sapwood discoloration, a few were striking examples. For the period June 17 to October 15, 1957, tree 8 had a sapwood moisture content that was lower than the average of all inoculated trees. This tree exhibited 10% wilt on July 1, 1957, and 50% on August 12, 1957, whereas the length of sapwood discoloration was 10½ in. Tree 15, which had a slightly higher water content than tree 8, but lower than the average, showed 20% and 70% wilting for the respective dates. The extent of discoloration in this tree was 13 in.

Under the conditions of this experiment a definite relationship was shown between sapwood moisture content and severity of wilt symptoms and extensiveness of sapwood discoloration. The data show that both of the latter phenomena are decreased with an increase in sapwood water content. However, since these observations were made under field conditions, confirmation is needed under controlled conditions of temperature, precipitation, and soil moisture.

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TAXONOMY OF THE PERONOSPORA SPECIES ON CRUCIFERAE AND CHENOPODIACEAE[1, 2]

William D. Yerkes, Jr.,[3] and Charles Gardner Shaw[4]

SUMMARY

Numerous specimens on each of several host species within the *Peronospora* complexes on Cruciferae and Chenopodiaceae were compared morphologically. Within each complex the oospores were remarkably uniform in structure. Variation in conidiophore structure within a single specimen and in series of specimens from one host species prevented the association of conidiophore types with host species or genera. Mean conidial measurements (length and width) for different collections of *Peronospora* on one host often varied by several microns. The range in means of conidia from a series of collections on a single host included the means obtained from other host species of the same family. There was insufficient agreement among the conidial measurements obtained to consider either the means for length and width or their quotient significant.

In the Peronosporaceae neither morphology nor physiologic specialization alone provides a satisfactory basis for species delimitation. It is proposed that physiologic specialization be utilized in conjunction with, but subordinate to, morphology. When downy mildews assignable to the same genus occur on hosts of one family and differ morphologically without intergradation, these entities constitute distinct species. If, however, morphologic variation on a single host includes the variations found on other hosts of the same family, only one species is recognized. Or, if morphologically the material from various hosts within one family forms a gliding, overlapping series, all of it is considered conspecific. This concept retains the species as the basic taxon, within which infraspecific taxa can be erected if necessary.

A single species, *Peronospora parasitica* (Fr.) Fr., is recognized on the Cruciferae, and but one species, *P. farinosa* (Fr.) Fr., on the Chenopodiaceae. Synonymy of these two species is listed.

INTRODUCTION.—Following de Bary's (3) treatment of the family "Peronosporei," Schroeter (36, p. 228-252; 37), Fischer (11), and Berlese (4), in Europe, and Farlow (8, 9), Swingle (41, 42), and Wilson (50, 51, 52, 53, 54), in America, monographed the Peronosporaceae or major portions of the family.

During this period of about 60 years the generic and family limits of the Peronosporaceae became well established. Species were not characterized entirely on a morphological basis, but morphology remained a primary basis for species differentiation. Each monographer treated the group conservatively, recognizing that variation within a species might be considerable. Although many varietal and form names were utilized by distributors of exsiccati, few of these were validly published (25).

An entirely different basis for species differentiation was employed by Gäumann (13), who studied the physiology and morphology of *Peronospora parasitica* (Fr.) Fr. His inoculation studies produced evidence of numerous pathogenic forms or races, each with limited host range, within this complex. The morphology of 3 structures—the oospores, the conidiophores, and the conidia (especially their dimensions)—was then checked. Oospores were considered of no value for systematic purposes, but quite different conclusions were reached about the conidiophores and conidia. Although transition occurred, and integradation through all sorts of intermediate forms was observed, 15 types of conidiophores were distinguished on the basis of total height, thickness of the swollen base, the structure of the ultimate branches, etc. Of all these, the form of the ultimate branches was considered most decisive and the only one not influenced by environment.

One thousand measurements were made of the length and width of conidia on 80 different specimens, each collected on a different cruciferous host. The averages varied in a gliding series, from 15.17×13.11 μ for conidia on *Erophila verna* (L.) E. Mey., to 27.81×24.16 μ for conidia on *Nasturtium montanum* Wall. The possibility that these measurements might indicate a single, but variable, species was discussed and discarded, since the conidia from one host differed from those obtained from most other hosts in one respect or another.

The following general principles were set up (13, p. 514) for limiting species in the genus *Peronospora:* "If two *Peronosporae* possess essentially the same oogonia and oospores, then they belong to the same section (*Gruppe*); if they agree in the important traits of the conidiophores, they belong to the same subsection (*Unterabteilung*); and if they agree in their conidia, then they belong to the same species. However, if the conidia differ in form and size or if in-

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The studies of the *Peronospora* on the Chenopodiaceae are based on a portion of a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, State College of Washington, by the senior author; those on the *Peronospora* on the Cruciferae are based on a portion of a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, University of Wisconsin, by the junior author.

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dividual differences are evident in the case of other organs, then two separate species must be established. . . . If biological specialization occurs simultaneously, actually in the final analysis, the name of the host suffices for an unequivocal determination. . . Thus, in this way, we reach the point where the original characters, namely the morphological ones, are shoved aside in favor of the biological characters" (free translation).

Applying these principles to the material studied, Gäumann split *P. parasitica* into 52 species, 49 of which were new. In later papers (14, 16, 17), other *Peronospora* complexes, including *P. farinosa* (Fr.) Fr. on the Chenopodiaceae (15), were investigated by Gäumann and treated in similar fashion. In his monograph of the genus *Peronospora*, Gäumann (18) recognized 268 species, more than half erected by him and his co-workers.

Only one instance could be found in Gäumann's papers where conidial averages were computed for 2 collections made on the same host species. The averages of material collected by Gäumann (13, p. 505-6) on *Capsella bursa-pastoris* (L.) Medic. were 20.87×18.21 μ, the quotient (average length divided by average width) being 1.15; the averages of conidia from a specimen on the same host in Sydow's exsiccatum, *Phycomycetes et Protomycetes* #67, were 21.9×18.3 μ, the quotient being 1.20. Average lengths for conidia from 3 other hosts, referred to 3 different *Peronospora* species, fall between 20.87 μ and 21.9 μ. Average widths for conidia from 2 other hosts, referred to 2 different *Peronospora* species, fall between 18.21 μ and 18.3 μ. The quotients of conidia from 26 different hosts, assigned to 18 different *Peronospora* species, either coincide with or fall between 1.15 and 1.20. According to Gäumann (13, p. 435), the differences between the conidial averages of these two collections on *C. bursa-pastoris* were due to one's being immature.

Conclusions different from those of Gäumann were reached by 2 of his contemporaries. Schweizer's (38) morphologic studies of *Bremia lactucae* Regel were based on numerous specimens from each host species. Although finding biological specialization within *B. lactucae*, the morphological studies led to the conclusion that the great differences encountered in spore sizes are not absolute morphological species characteristics, but are due to other factors (e.g., moisture and host influence). Wartenweiler (49), after studying the morphology of 3 species of *Plasmopara*, deplored the endless splitting of fungus species and emphasized the importance of the species as the common systematic unit.

Nevertheless, Sydow erected 3 new species of *Bremia* (43, 44), substantiating them by saying that Schweizer had proved their host specificity! Besides Sydow, Savulescu and Rayss (34) and Oescu and Radulescu (30), in Roumania, Rayss, in Palestine (31), Ito (23) and Sawada (35), in Japan, and others have described new species almost every time a downy mildew has been found on a previously unreported host. In but few instances have inoculations been performed to substantiate the supposed host specificity.

However, several workers, including Thung (46) and Hammarlund (19), on *Peronospora parasitica*, Richards (32), on *P. spinaciae* Laub., Angell and Hill (1) and Clayton and Stevenson (7), on *P. tabacina* Adam, Uppal and Weston (47), on *Sclerospora philippinensis* Weston, and Ling and Tai (27), on *Bremia lactucae*, have demonstrated considerable morphologic variability within single species of the Peronosporaceae. These studies indicate that the morphology of the conidia and conidiophores is modified by temperature, moisture, season, and other factors.

Investigations involving the study of numerous downy mildew collections on the same host have raised doubts as to the validity of Gäumann's concepts. Consequently, a study of numerous collections on each of several hosts within 2 *Peronospora* complexes was undertaken to determine the variability in oospores, conidia, and conidiophores occurring naturally on individual hosts and to ascertain the justification of assigning a single set of mean values for the length and width of conidia from a particular host and using these mean values as diagnostic characters in the delimitation of species. The two complexes selected for this investigation were those on the Chenopodiaceae (the *P. farinosa* complex) and the Cruciferae (the *P. parasitica* complex).

Methods.—Material to be examined was flooded with 70% ethyl alcohol, mounted on glass slides in 5% KOH, and warmed gently. Only detached conidia that were fully turgid and so aligned that the axis of greatest length was parallel to the optical plane were measured. Since different numbers of spores were measured in studying the two complexes, these details are presented in the discussion of each section of the work. Conidiophore characters were ascertained from the same mounts utilized for spore measurements. Several mounts were made from each collection in an attempt to find oospores in as many hosts as possible. Necrotic portions of lesions were checked carefully for oospores.

The Peronospora complex on the Cruciferae.—Abundant material on *Capsella bursa-pastoris* being available in the herbarium at the University of Wisconsin, measurements were made of 100 spores from each of 13 specimens collected at various times during the growing seasons (May, June, and July) from 1913 through 1946. Conidial means for these specimens ranged from 20.79×15.86 μ to 28.40×19.70 μ, a range that includes more than half the total range for means (15.17×13.11–27.81×24.16 μ) found by Gäumann (13) on 80 different cruciferous hosts. Additional conidial averages were then computed on other cruciferous hosts, the customary procedure being to measure 100 spores from the first specimen examined on each different host and 20 spores from any additional specimens studied on that host.

These spore measurements are summarized in Table 1. Gäumann's spore measurements, means, and quotients are included in those instances where he studied material from the hosts studied by us. It is apparent

that mean spore measurements from 2 or 3 collections of *Peronospora* on the same host often vary by several microns. Comparable variation also occurs as often as not between the conidial means obtained by Gäumann and by ourselves for the same host. Since particular importance has been attached to the quotient (i.e., mean length/mean width), it is interesting to note that this figure also shows divergence when computed for 2 or 3 collections on the same host.

These results indicate: 1) that the conidia of *Peronospora* vary significantly in size from collection to collection on the same host, and 2) that there is not sufficient agreement among the measurements obtained by different workers on different collections from the same host to permit the assignment of special significance to either the means or the quotient.

While conidial measurements can be averaged and briefly presented in tabular form, any adequate description of the variability in conidiophores would require many pages.

Although Gäumann (13) reported definite conidiophore types on certain hosts, we did not find distinct conidiophore types regularly associated with particular hosts or host genera. Prior to Gäumann, Wilson (53)

TABLE 1.—*Collections of conidial measurements of Peronospora on Crucifera*

	Length (μ)				Quotient (length/width)			Width (μ)				No. spores measured + no. collections [a]
	Means			Range	Means			Means			Range	
	Min.	Grand	Max.		Min.	Grand	Max.	Min.	Grand	Max.		
Sisymbrium altissimum L.		(15.66)		11.9–19.9		(1.32)			(11.89)		9.6–15.3	100 (1)
Arabis hirsuta [b]		(16.12)		11.0–23.0		(1.16)			(13.92)		8.0–20.0	1,000 (1)
Arabis laevigata (Muhl.) Poir.		(16.89)		10.3–16.1		(1.24)			(13.63)		12.6–19.9	100 (1)
Arabis hirsuta (L.) Scop.	16.99	(17.62)	18.11	13.4–22.6	1.23	(1.30)	1.37	13.02	(13.54)	14.48	11.1–19.2	220 (3)
Cardamine bulbosa (Schreb.) BSP.		(17.72)		13.4–23.0		(1.25)			(14.22)		11.5–16.1	100 (1)
Draba caroliniana [b]		(17.64)		11.0–24.0		(1.12)			(15.81)		9.0–21.0	1,000 (1)
Rorippa palustris (L.) Bess.		(18.75)		15.3–24.9		(1.31)			(14.27)		11.5–18.0	100 (1)
Cardamine parviflora L.		(18.90)		14.2–23.0		(1.28)			(14.79)		11.5–17.2	100 (1)
Nasturtium officinale R. Br.	18.32	(19.50)	20.29	14.6–26.8	1.17	(1.21)	1.25	15.33	(16.19)	17.37	12.3–21.1	140 (3)
Cardamine pennsylvanica Muhl.		(19.62)		15.3–23.8		(1.35)			(14.56)		12.6–16.9	100 (1)
Brassica nigra (L.) Koch		(19.77)		15.7–23.7		(1.16)			(17.00)		14.5–21.4	100 (1)
Brassica arvensis (L.) Ktze.		(19.97)		15.3–27.2		(1.25)			(16.04)		11.5–20.7	100 (1)
Raphanus sativus [b]		(20.01)		14.0–26.0		(1.10)			(18.17)		14.0–22.0	1,000 (1)
Draba caroliniana Walt.	19.53	(20.19)	21.37	14.6–26.4	1.21	(1.22)	1.25	15.68	(16.58)	17.66	11.9–20.7	220 (3)
Nasturtium officinale [b]		(20.32)		16.0–27.2 [c]		(1.19)			(17.07)		12.8–20.8 [c]	101 (1)
Dentaria laciniata Muhl.	20.23	(21.17)	22.87	15.3–27.6	1.26	(1.29)	1.31	15.69	(16.45)	17.41	11.5–21.8	140 (3)
Capsella bursa-pastoris [b]	20.87	(21.39)	21.90	12.0–35.0	1.15	(1.18)	1.20	18.21	(18.26)	18.30	11.0–24.0	1,200 (2)
Capsella bursa-pastoris (L.) Medic.	20.79	(23.10)	28.40	14.6–36.8	1.26	(1.35)	1.44	15.86	(17.14)	19.70	11.9–23.8	1,200 (13)
Raphanus sativus L.		(23.54)		18.0–29.5		(1.26)			(18.72)		14.2–22.6	100 (1)
Sisymbrium canescens [b]		(26.38)		17.0–32.0		(1.36)			(13.25)		12.0–24.0	1,000 (1)
Sisymbrium canescens Nutt.		(27.13)		19.9–33.3		(1.77)			(15.33)		11.9–18.4	100 (1)
Lepidium virginicum [b]		(27.52)		19.0–37.0		(1.50)			(18.35)		14.0–24.0	1,000 (1)
Lepidium apetalum Willd.	28.94	(29.19)	29.38	19.1–38.3	1.46	(1.47)	1.48	19.44	(19.79)	20.14	15.3–25.3	120 (2)
Lepidium virginicum L.		(29.46)		23.0–38.3		(1.50)			(19.68)		15.7–26.8	100 (1)

[a] 100 spores measured from one or two collections on each host, and from all collections on Capsella bursa-pastoris; 20 spores measured from remaining collections.

[b] Measurements from Gäumann (13).

[c] Range in spore size taken from Fig. 28 & 29 (Gäumann, 13); figures in desc. (op. cit., p. 528) incorrect.

recognized 3 species within the *Peronospora* complex on crucifers, and attempted to segregate them on morphologic characters. Six different host genera were listed for *P. lepidii* (McAlp.) G. W. Wils., which was characterized as having relatively simple conidiophores, with ascending branches not forming a dense head. We have frequently encountered conidiophores of this type on *Lepidium apetalum* Willd. and *L. virginicum* L. However, much-branched conidiophores with dense tangled heads (typical of *P. parasitica* sensu Wilson) occur exclusively in some specimens on *L. apetalum*. Also, some collections on *L. virginicum* show considerable variation and contain numerous intermediate types. Variation in size and structure of the conidiophores, both in single collections and in series of collections on 1 host species, was such that we were unable to associate conidiophore types with either particular host genera or host species.

Oospores were found in 10 of the 17 hosts listed in Table 1. As reported by Gäumann (13), the oospores are remarkably uniform in structure, regardless of the cruciferous host on which they are found. The thickness of the oogonial wall is marked in collections with mature oospores. The exospore is thin compared to that of other *Peronospora* species, and is superficially smooth, although the oospore may be somewhat angular in outline.

The conidial measurements, our inability to associate conidiophore types with particular host genera, and the uniformity of the oospores convince us that there is no reliable morphological basis for distinguishing species within the *Peronospora* complex on cruciferous hosts. We conclude that a single species, *Peronospora parasitica* (Fr.) Fr., should be recognized on cruciferous hosts. Although extensive, the list of synonyms given below includes every species of *Peronospora* known to have been described on a cruciferous host. If more than a single species is to be recognized, apparently only the number of species within the Cruciferae capable of being infected by some *Peronospora* strain will limit the number of *Peronospora* species that must eventually be recognized within this single complex.

PERONOSPORA PARASITICA (Pers. ex Fr.) Fr., Sum. Veg. Scand.: 493. 1849.

The synonyms of *P. parasitica* (Pers. ex Fr.) Fr. are: *Botrytis parasitica* Pers., Obs. Myc. 1: 96. T. 5, fig. 6. 1796; *Mucor botrytis* Sow., Eng. Fungi 3: T. 359. 1802; *M. erysimi* Sow., Eng. Fungi 3: T. 400, fig. 7. 1803; *Botrytis nivea* Mart., Fl. Crypt. Erlang.: 342. 1817; *B. parasitica* Pers. ex Fr., Sys. Myc. 3: 404. 1832. (*BASONYM*); *Perenospora parasitica* Fr., Sum. Veg. Scand.: 493. 1849; *Peronospora parasitica* (Pers.) Tul., Compt. Rend. Acad. Sci. 38: 1103. 1854; *P. ochroleuca* Ces. in Rab., Herb. Myc., ed. II, #175. 1855 (also Bot. Zeit. 14: 87. 1856.); *P. dentariae* Rab., Fungi europ. II, #86. 1859 (also Flora 42: 436. 1859.); *P. parasitica* Pers. ex d By., Ann. des Sci. Nat. (IV) 20: 117. 1863; *P. botrytis* (Sow.) Cocc. & Mor., Mem. Accad. Sci. Ist Bologna (IV) 6: 394. (1884). 1885; *P. parasitica* d By. var. *lepidii* McAlp., Proc. Royal Soc. Victoria, N. S. 7: 221. 1895; *P. niessleana* Berlese, Icon. Fung. Phyc. 40, pl. 66, fig. 1. 1898; *P. lepidii* (McAlp.) G. W. Wils., Mycologia 6: 198. 1914; *P. alliariae wasabi* Gäum., *P. alyssi calycini* Gäum., *P. alyssi incani* Gäum., *P. arabidis alpinae* Gäum., *P. arabidis glabrae* Gäum., *P. arabidis hirsutae* Gäum., *P. arabidis oxyphyllae* Gäum., *P. arabidis turritae* Gäum., *P. arabidopsidis* Gäum., *P. barbaraeae* Gäum., *P. berteroae* Gäum., *P. biscutellae* Gäum., *P. brassicae* Gäum., *P. buniadis* Gäum., *P. calepinae* Gäum., *P. camelinae* Gäum., *P. cardamines laciniatae* Gäum., *P. cheiranthi* Gäum., *P. chorisporae* Gäum., *P. conringiae* Gäum., *P. coronopi* Gäum., *P. dentariae macrophyllae* Gäum., *P. diplotaxidis* Gäum., *P. drabae* Gäum., *P. erophilae* Gäum., *P. erucastri* Gäum., *P. erysimi* Gäum., *P. gäumanniana* Jaap. ex. Gäum., *P. hesperidis* Gäum., *P. isatidis* Gäum., *P. lepidii sativi* Gäum., *P. lepidii virginici* Gäum., *P. lunariae* Gäum., *P. matthiolae* Gäum., *P. nasturtii aquatici* Gäum., *P. nasturtii montani* Gäum., *P. nesleae* Gäum., *P. roripae islandicae* Gäum., *P. sisymbrii intermediae* Gäum., *P. sisymbrii loeselii* Gäum., *P. sisymbrii officinalis* Gäum., *P. sisymbrii orientalis* Gäum., *P. sisymbrii sophiae* Gäum., *P. sophiae pinnatae* Gäum., *P. teesdaleae* Gäum., *P. thlaspeos alpestris* Gäum., *P. thlaspeos arvensis* Gäum., *P. thlaspeos perfoliati* Gäum., and *P. turritidis* Gäum., Beihefte Bot. Cent. 35: 519-531. 1918; *P. rhaetica* Gäum., *P. norwegica* Gäum., *P. sophiae intermediae* Gäum., and *P. cochleariae* Gäum., Beitr. z. KryptogamenFl. der Schweiz 5(4): 251, 252, 276, 280. 1923; *P. iberidis* Gäum., Ann. Myc. 25: 176. 1927; *P. calycini* Hruby, Hedw. 68: 158. 1928. (*Nomen Nudum*); *P. alyssi arduini* Hruby and *P. alyssi montani* Hruby, Hedw. 69: 184. 1929; *P. rapistri* Jacz. & Serguieva, Opriedielitel Gribov soverscheniîe gribîi (Diploidniie stadii), Tom. I. Fikomitzetî: 143. 1931; *P. lepidii perfoliati* Savul. et Rayss and *P. euclidii* Savul. et Rayss, Ann. Myc. 30: 367, 369. 1932; *P. rapistri Oescu* et Radul., Ann. Sci. l'univ. Jassy 18: 434. 1933; *P. maublancii* Savul. et Rayss, Bull. Soc. Myc. France 50: 93. 1934; *P. nasturtii-palustris* Ito & Tokunaga, Trans. Sapporo Nat. Hist. Soc. 14: 31. 1935; *P. eigii* Rayss, Palest. J. Bot., J. Ser., 1: 154. 1938; *P. galligena* Blumer, Mitt. Naturf. Ges. Bern 1937: 17. 1938; *P. iranica* Petr. & Esfandiari, Ann. Myc. 39: 204. 1941; *P. takahashii* Ito apud Ito & Murayama, Trans. Sapporo Nat. Hist. Soc. 17: 162. 1943; *P. coronopi-procumbentis* Vien.-Bourg. and *P. minuta* Vien.-Bourg., Bull. Soc. Myc. France 69: 333. 1954; *P. cakiles* Savile, Can. Jour. Bot. 35: 197. 1957.

THE PERONOSPORA COMPLEX ON THE CHENOPODIACEAE.—Ninety-two collections of *Peronospora* were studied on 8 different host species of the Chenopodiaceae. Four collections on unidentified species of *Chenopodium* were also included and treated as a unit. The precautions mentioned under Methods were observed in measuring 25 conidia from each of these collections. Table 2 summarizes the measurements of conidial length and width obtained. As in the *Peronospora* complex on the Cruciferae, attempts were made

prior to Gäumann's studies (15) to distinguish species and infraspecific taxa within the *Peronospora* forms that occur on members of the Chenopodiaceae. Caspary (6) recognized 2 forms, var. *major* and var. *minor*. This segregation was used by several subsequent workers. The two forms might be characterized as follows:

var. *major*—Ultimate branches short, stout, reflexed; conidia usually pedicellate.

var. *minor*—Ultimate branches narrower, straighter; conidia not distinctly pedicellate.

Wilson (53) recognized both *P. effusa* (Grev.) Ces. (equals *P. effusa* var. *major* Casp.) and *P. farinosa* (Fr.) Keissl. [equals *P. effusa* var. *minor* Casp.; evidently both Keissler and Wilson overlooked the combination *P. farinosa* (Fr.) Fr.]. That Wilson lists *Chenopodium album* L., *C. hybridum* L., and *Spinacia oleracea* L. as hosts for both species indicates the difficulty encountered in attempting to distinguish species within the complex.

Our study discloses that, not only do both forms occur on the three hosts mentioned but intermediate forms are found on all hosts, and occasionally a single specimen will show almost the complete range in variation. Not only do the ultimate branches vary, but there is no consistency in the presence or absence of a basal pedicel on the conidia. Conidial pedicels were present in some collections from each of the hosts studied.

The oospores present on chenopodiaceous hosts offer no characters upon which to make distinctions. In the nine hosts studied, oospore diameter varied from 20 to 50 μ, there being no correlation between host species and oospore size.

That conidial measurements are not a satisfactory criterion for species separation is indicated by the figures in Table 2. For example, the mean lengths of conidia from different collections of *Peronospora* on *Chenopodium album* (the host on which the largest number of collections was available) include within their extremes the mean conidial lengths of all other collections except for the five collections on *Beta vulgaris* L. with the smallest conidia. Similarly, the mean widths of conidia from different collections on *C. album* include within their extremes mean conidial widths of all other collections except for the two collections on *C. hybridum* with the smallest conidia and the collection on *B. vulgaris* with the smallest conidia.

Being unable to ascertain any suitable or reliable morphological differences within the *Peronospora* complex on chenopodiaceous hosts, we conclude that, in this complex also, only a single species should be recognized, namely, *Peronospora farinosa* (Fr.) Fr. The synonymy of this species, which is not as extensive as that of *P. parasitica*, follows.

PERONOSPORA FARINOSA (Fr.) Fr., Sum. Veg. Scand.: 493. 1849.

The synonyms of *P. farinosa* (Fr.) Fr. are: *Botrytis epiphylla* Pers., Myc. Eur.: 77. 1822; *B. effusa* Grev., Fl. Edin.: 468. 1824; *B. farinosa* Fr., Sys. *Myc.* 3: 404. 1832. (*BASONYM*); *B. effusa* Grev. ex Desm., Ann.

TABLE 2.—*Conidial measurements of collections of Peronospora on Chenopodiaceae*

	Length (μ)				Quotient (length/width)			Width (μ)				No. spores measured + no. collections[a]
	Means			Range	Means			Means			Range	
	Min.	Grand	Max.		Min.	Grand	Max.	Min.	Grand	Max.		
Spinacia oleracea[b]		(24.30)		17.6–32.0		(1.22)			(19.90)		12.8–25.6	500 (1)
Beta vulgaris L.	21.54	(24.81)	27.69	17.5–32.5	1.25	(1.30)	1.36	17.13	(18.94)	20.88	13.7–23.7	375 (15)
Chenopodium murale[c]		(24.83)		16.0–32.0		(1.14)			(21.63)		12.8–28.8	500 (1)
Chenopodium hybridum[b]		(24.98)		17.6–32.0		(1.46)			(17.11)		8.0–24.0	500 (1)
Chenopodium bonus-henricus[b]		(26.01)		17.0–34.0		(1.13)			(22.86)		16.0–31.0	500 (1)
Chenopodium sp.	24.88	(26.55)	28.72	20.0–35.0	1.30	(1.34)	1.40	19.09	(20.09)	20.46	16.2–26.2	100 (4)
Chenopodium leptophyllum Nutt.		(26.68)		20.0–33.7		(1.38)			(19.28)		15.0–23.7	25 (1)
Spinacia oleracea L.	24.43	(26.92)	28.99	20.0–37.5	1.26	(1.37)	1.47	18.08	(19.61)	20.74	15.0–25.0	750 (30)
Chenopodium bonus-henricus L.	26.23	(26.95)	27.67	22.5–33.7	1.28	(1.30)	1.33	20.43	(20.70)	21.32	16.2–23.7	125 (5)
Chenopodium hybridium L.	23.94	(27.53)	33.44	16.9–40.2	1.30	(1.38)	1.50	17.11	(19.88)	22.23	15.3–25.7	150 (6)
Chenopodium gigantospermum Aeller		(28.58)		26.2–36.5		(1.46)			(19.57)		17.5–21.2	25 (1)
Chenopodium murale L.		(29.09)		25.0–35.0		(1.31)			(22.17)		16.2–26.2	25 (1)
Chenopodium album[b]		(29.10)		17.2–40.2		(1.53)			(19.04)		12.2–27.2	500 (1)
Chenopodium album L.	23.83	(29.65)	33.53	20.0–40.0	1.31	(1.41)	1.58	17.77	(20.98)	23.17	14.6–27.5	825 (33)

[a] 25 spores measured from each collection by authors; 500 spores measured by Gäumann.
[b] Measurements from Gäumann (15).
[c] Measurements from Gäumann (18).

des Sci. Nat. (II) 8: 5; T. 1, fig. 1. 1837. (*pro parte*); *Peronospora nivea* Ung., Bot. Zeit. 5: 314. 1847. (*p. p.*); *Perenospora farinosa* Fr., Sum. Veg. Scand.: 493. 1849; *Peronospora chenopodii* Schlecht. and *Monosporium chenopodii* Schlecht., Bot. Zeit. 10: 619. 1852; *Peronospora chenopodii* Casp., Bot. Zeit. 12: 565. 1854; *P. effusa* Ces. in Rab., Herb. Myc., ed. I. #1880. 1854 (also Bot. Zeit. 12: 190. 1854.); *P. effusa* Rab. *α major* Casp. and *P. effusa β minor* Casp., Monatsber. d. Kgl. Preuss, Akad. d. Wissensch. Berlin 1855: 330. 1855; *P. effusa* [Grev.] d. By., Ann. des Sci. Nat. (IV) 20: 115. 1863; *P. schachtii* Fckl., Fung. rhen. #1508. 1865 (also Symb. Myc.: 71. 1869.); *P. betae* J. Kuehn, Zeitschr. Landw. Cent. Prov. Sachsen 29: 276. 1872; *P. epiphylla* (Pers.) Pat. and Lagerh., Bull. Soc. Myc. France 7: 167. 1891; *P. spinaciae* Laub., Gartenflora 55: 464. 1906; *P. farinosa* (Fr.) Keissl., Ann. d. K. K. Naturh. Hofm. Wein 25: 229. 1911; *P. vistulensis* Wrób., Bull. Acad. Sc. Cracovie, ser. B., 1915: 243. 1915; *P. chenopodii-ficifolii* Saw., Agr. Exp. Sta. Formosa Cir. Pub. 101: 9-10, 15; figs. 6-10. 1916 (also T. Tan., Mycologia 11: 83. 1919.); *P. boni-henrici* Gäum., *P. chenopodii glauci* Gäum., *P. chenopodii polyspermi* Gäum., *P. chenopodii rubri* Gäum., *P. kochiae* Gäum., *P. litoralis* Gäum., *P. minor* (Casp.) Gäum., and *P. variabilis* Gäum., Mitteil. Naturf. Gesellsch. Bern 1918: 62-64. 1919; *P. muralis* Gäum., *P. bohemica* Gäum., *P. monolepidis* Gäum., and *P. amaranthi* Gäum., Beitr. z. KryptogamenFl. d. Schweiz 5(4): 223, 234, 235. 1923; *P. chenopodii opulifolii* Savul. et Rayss, *P. tatarica* Savul. et Rayss, *P. atriplicis hastatae* Savul. et Rayss, *P. atriplicis hortensis* Savul. et Rayss and *P. chenopodii urbici* Savul. et Rayss, Ann. Myc. 30: 357, 359, 360, 363, 365. 1932; *P. nitens* Oescu et Radul. and *P. atriplicis tataricae* Oescu et Radul., Ann. Sci. de l'univ. Jassy 18: 427. 429. 1933; *P. atriplicis halimi* Savul. et Rayss, Bull. Soc. Myc. France 50: 90. 1934; *P. chenopodii vulvariae* Savul. et Rayss and *P. obionis verruciferae* Savul. et Rayss, Ann. Myc. 33: 1, 3. 1935.

Discussion.—Although biologists almost universally accept the species as the fundamental taxonomic unit, the lack of agreement and understanding regarding what constitutes a species has been a major source of confusion. Hatch (20) defined a species as being "composed of those specimens which upon examination the taxonomist believes to be cospecific." The vagueness of this definition is characteristic of most attempts to delimit the taxon in question.

Organisms at various evolutionary levels differ so greatly that specialists in different groups inevitably develop their own species concepts. Bacterial species, for example, are defined primarily on biochemical characters, whereas, in the Angiosperms, morphological characters are employed almost exclusively. Mycologists have utilized physiology, supplemental to morphology, to delimit species primarily among the parasitic fungi. Too often physiologic (host) specialization has been assumed to exist and considered a useful criterion for distinguishing species, without considering the degrees of physiologic specialization or types of parsitism that exist in different groups of the fungi.

For the purposes of this discussion, the parasitic fungi may be divided into 2 groups, the obligately parasitic fungi and the non-obligate parasites, which at some state in their life cycles characteristically live as facultative saprophytes. Among the obligately parasitic fungi, host specialization has obviously developed to a much higher degree than in most non-obligately parasitic fungi. In the Uredinales, Ustilaginales (strictly speaking, and depending partially on definition, the Ustilaginales are not obligate parasites, but nevertheless they exhibit a high degree of parasitism), Peronosporaceae, and Erysiphales, cross-inoculation studies have repeatedly demonstrated differences in host specificity for morphologically similar isolates. The type of parasitism exhibited by these fungi supports the hypothesis that these fungi have evolved in intimate association with their respective hosts, becoming progressively more highly specialized in their parasitism and more limited as to host range. In such groups it seems justifiable to utilize physiologic specialization in conjunction with, but still subordinate to, morphology in delimiting species (12).

The host ranges of the majority of the non-obligately parasitic fungi have not been ascertained. Mycologists, perhaps by analogy with the obligate parasites, apparently have often assumed a higher degree of host specificity in these fungi than actually exists, and consequently have described large numbers of species in genera such as *Alternaria*, *Cercospora*, *Colletotrichum*, *Fusarium*, *Phoma*, *Phyllosticta*, *Septoria*, etc. Cross-inoculations and cultural studies such as those of Johnson and Valleau (24), on *Cercospora*, Snyder and Hansen (39), on *Fusarium*, Neergaard (28), on *Alternaria*, and Tiffany and Gilman (45), on *Colletotrichum*, indicate that host specialization is more supposed than actual. Such studies reaffirm the importance of morphologic characters in delimiting species in these groups of the fungi. That relatively few morphologic species exist in such genera is indicated by von Arx's (2) recent treatment of *Colletotrichum*, in which only 11 species are recognized.

The premise that criteria may vary in their taxonomic significance in different groups of the fungi is actually strengthened if the criteria found useful in one group of the fungi prove unsatisfactory when applied to another group of the fungi exhibiting a different type of parasitism. Such is the case when the criteria suggested by Fischer and Shaw (12) are applied to a genus such as *Colletotrichum* (45).

Let us now consider what course should be followed in a taxonomic treatment of the Peronosporaceae, a family of obligate parasites. Three possible approaches are discussed below. The authors' decision regarding which of these was the proper one to adopt has been strongly influenced by the various considerations set forth above.

If biological specialization is accepted as a satisfactory criterion for distinguishing species in the obligately parasitic fungi, and if one wishes to assume that

this phenomenon, which has been demonstrated most clearly in the *Peronospora parasitica* complex (13), occurs in much the same manner throughout the Peronosporaceae, it is merely necessary to coin a new binomial each time a downy mildew is found on a new host species. Publication of a description giving the host binomial and sufficient characters to indicate the generic position of the downy mildew would seem adequate for effective and valid publication under this procedure. More complete descriptions, giving average spore measurements and attempting to describe the structure of the conidiophores in detail, almost certainly would have to be revised the next time the fungus was studied on this host, particularly if the first description was based on a single specimen.

The authors object to a taxonomic treatment of the Peronosporaceae that places such emphasis on biological specialization. In at least one instance among the downy mildews, relatively little host specificity has been demonstrated. Hoerner (22) showed that *Pseudoperonospora humuli* (Miy. & Tak.) G. W. Wils., besides infecting many varieties of cultivated hops (*Humulus lupulus* L.) and *H. japonicus* Sieb. & Zucc., can also infect species of *Cannabis*, *Celtis*, and *Urtica* [See also Salmon and Ware (33)]. Hoerner suggested that *P. urticae* (Lib. ex Berk. & Br.) Salm. & Ware, *P. cannabina* (Otth) Cruzi, *P. celtidis* (Waite) G. W. Wils., and *P. humuli* may all be physiologic races of one species.

Secondly, the host species cannot be considered the unit that determines host specialization. Gäumann (13) himself reported infection of *Brassica rapa* as well as *B. oleracea* with conidia from the latter. Furthermore, the existence of both susceptible and resistant varieties within many host species and the variation exhibited by different isolates of a single parasite indicate variability of both parasites and hosts below the species level. Whether infection will or will not result from a given inoculation would seem to depend rather on the genotypes of the host plants inoculated, and the genotype of the particular strain of the pathogen being used (as well as on the environal conditions). In a number of species of the rusts (40) and powdery mildews (29), experiments on host specialization have progressed to the point where distinct races of a given parasite can be identified on the basis of their pathogenicity to a small number of varieties of the host. As yet, however, little work of this nature has been done with the Peronosporaceae. Felton (10) pointed out that the forms of *Peronospora parasitica* on cabbage studied by him do not coincide with either those of Gäumann (13) or with those of Hiura and Kanegae (21). The demonstration of heterothallism, as well as homothallism and intermediate conditions, by de Brüyn (5), in *P. parasitica* on cabbage, indicate genotypic variation of the parasite. Wang (48) established 3 varieties within *P. parasitica;* viz. *brassicae* on *Brassica*, *raphani* on *Raphanus*, and *capsellae* on *Capsella*. Within *P. parasitica* var. *brassicae* he differentiated 6 races, distinguishable by their effects on ordinary cabbage (*B. oleracea* var. *capitata* L.), Chinese cabbage (*?B. pekinensis* Rupr.), *B. juncea* Coss., and swede (*B. napobrassica* Mill.).

In another species, *P. manshurica* (Naum.) Syd., Lehman (26) recently distinguished 4 new races, bringing to 6 (plus 2 subraces) the total number of races recognized within this species. These races and subraces can be distinguished from one another by the reactions to infection of 10 differential varieties of the host.

Finally, such a treatment does, of necessity, lead to the adoption of essentially a trinomial system of nomenclature at the species level. Many of the names erected by Gäumann, Savulescu, Rayss, and others are trinomials, e.g., *P. lepidii sativi* Gäum. (13), and *P. rumicis rosei* Rayss (31). As the hyphen was *consistently* omitted, it can be argued that "the Linnaean system of binary nomenclature for species was not consistently employed" [25, Art. 70(5)].

At the opposite extreme, a second possible method of treatment would reduce the number of species in the Peronosporaceae to a very few. If species were based on morphologic differences alone, the number of species would be reduced to less than half the number treated by de Bary (3) almost a century ago. This type of treatment, which has yet to be attempted, is considered almost as impractical as that based solely on host specialization. The chances of its being accepted by other workers seem remote indeed. The main objection to it is that it overlooks entirely the fact that the group under consideration does consist of obligate parasites. In the absence of consistent morphologic differences, those "strains" found by inoculation studies to be limited to a single host species are justifiably considered to be more closely related to one another than to "strains" occurring on other species of the same host genus; those occurring on species of the same host genus may be considered more closely related to one another than those occurring on species of different genera of the same host family; and finally those "strains" occurring on host species within one host family may be considered more closely related to one another than to "strains" occurring on species belonging to other families of hosts. The latter statement is justifiably derived from the hypothesis that obligate parasites have evolved together with their hosts, becoming progressively more highly specialized physiologically (both in their degree of parasitiism and their host specialization) but undergoing essentially no morphologic change. If, in groups of the fungi consisting of obligate parasites, species are differentiated solely on morphologic characters, one of the most obvious indicators of relationship is overlooked.

A third procedure, intermediate between the two discussed and rejected above, is recommended by the authors. We believe it yields a natural system of classification both for the Peronosporaceae and for other groups of the fungi consisting of obligate parasites or of parasites exhibiting a high degree of host specialization. At the same time it retains the species as the basic taxon, within which infraspecific taxa (varieties,

and forms or races) can be erected if considered necessary. The concept proposed is essentially the same as that proposed by Fischer and Shaw (12) for the Ustilaginales.

Arbitrarily the host family is taken into consideration. When downy mildews assignable to the same genus of the Peronosporaceae occur on hosts within the same family, but differ morphologically from one another, and when intergrading morphologic forms are not found, these entities are considered distinct species. If, however, the range of morphologic variation on a single host includes within its limits the variations found on other hosts of the same family, all material is referred to the same species. Or, if the material from various hosts within a single family appears to form a gliding, overlapping series, again it is referred to a single species.

Initially, of course, material from two different hosts belonging to the same family may appear distinct, particularly if only a single specimen from each host is available. The results of our studies, however, as well as those of Clayton and Stevenson (7) and others, indicate that, if the apparent differences are in the size and shape of the condia or conidiophores, the study of additional material on both these and other related hosts from numerous localities will usually disclose either a completely integrated series of minor variations or such extreme variation on one or all the hosts involved that the supposed diagnostic differences are no longer discernible.

The logical sequence to the adoption of such a species concept is the erection of infraspecific taxa within both *P. farinosa* and *P. parasitica*. Certainly, such taxa are needed within many species of the Peronosporaceae to indicate our present knowledge of host specificity. Infraspecific taxa are not proposed in this paper, because the nomenclatural problems involved in determining the correct epithets are considerable, and because it is believed that additional cross inoculation studies should precede such action.

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THE AIRBRUSH AS A TOOL IN VIRUS INOCULATIONS[1]

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SUMMARY

By using an airbrush, a refined type of atomizer, a 10- to 20-fold increase in efficiency of virus inoculations was achieved over conventional inoculation methods. Successful inoculations may thus be made with inoculum diluted 10- to 20-fold more than conventionally. This technique may be of value with viruses that occur in low titer. Variability in lesion counts between cotyledons is less with airbrush inoculations, and only ⅓ as many replicates are required for quantitative work. The optimum conditions for using the airbrush on cucumber cotyledons are: 1) 1-cm distance of airbrush nozzle to leaf surface, 2) 4 atmospheres air pressure, 3) inoculum delivery rate of 10 ml/min., 4) inoculation time of about 4 seconds to cover cotyledon completely, and 5) 1% by weight of 600-mesh carborundum in the inoculum.

Cucumber is a host for a number of viruses, and it is often convenient to use it as a local-lesion host for TMV (tobacco mosaic virus). Previous studies in this laboratory (2) have demonstrated the extreme inefficiency of conventional inoculation methods in producing local TMV starch lesions in cucumber cotyledons. The problem is similar to the one with necrotic local lesions in other hosts. With the inoculation methods used, it was necessary to injure some cells excessively in order to obtain a maximum number of lesions from the remaining cells. Among investigations toward improving inoculation efficiency was a study of application of inoculum by spraying techniques. Duggar and Johnson (1) were the first to use an atomizer for virus inoculations, and Richards and Munger (3) adapted this technique for large-scale inoculations. Herein is reported a method that greatly increases the efficiency of virus inoculations by spray application.

Method.—An airbrush is simply a refined type of atomizer; the one used in this work was a Paasche "H 3-in-1" artist's airbrush with an 80-ml glass reservoir. Methods of preparing inoculum and handling cucumber test plants were those used previously (2).

The important variables in airbrush inoculations are: 1) distance of airbrush nozzle from surface of test plant, 2) air pressure, 3) size of liquid orifice, 4) amount and type of abrasive, and 5) length of time test test leaf is sprayed. Each of these variables was investigated singly and in combination with other variables. Fig. 1 shows the effect of each variable on lesion counts with the other variables at an optimum for infection.

Since distance between the nozzle and the plant surface is critical, it was controlled by fitting a 1-hole

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cork over the nozzle so that the distance from the orifice to the end of the cork was exactly 1 cm. The air escape from the airbrush nozzle provided an "air cushion," so that the cork itself did not rub the cotyledon surface. The hole in the cork was flanged outward so as not to impede the flow of inoculum.

The size of the liquid orifice was calibrated in terms of rate of water delivery in ml/min. Flow for maximum lesions was optimum at about 10 ml/min., which was equivalent to 1¼ turns of the control knob.

Relatively few lesions were produced when inoculum was used without abrasive. It was hoped that, with enough pressure, the virus particle itself might serve as its own abrasive. However, this was not achieved with the equipment used. The 600-mesh carborundum proved more satisfactory than other abrasives tested. Celite clogged the orifice. Talc or calcium carbonate (precipitated) gave only about 10% as many lesions as carborundum.

Four passes of the airbrush, in a direction parallel to the mid-rib, were required to cover a cotyledon completely with inoculum. This required about 4 seconds, and gave the maximum number of lesions.

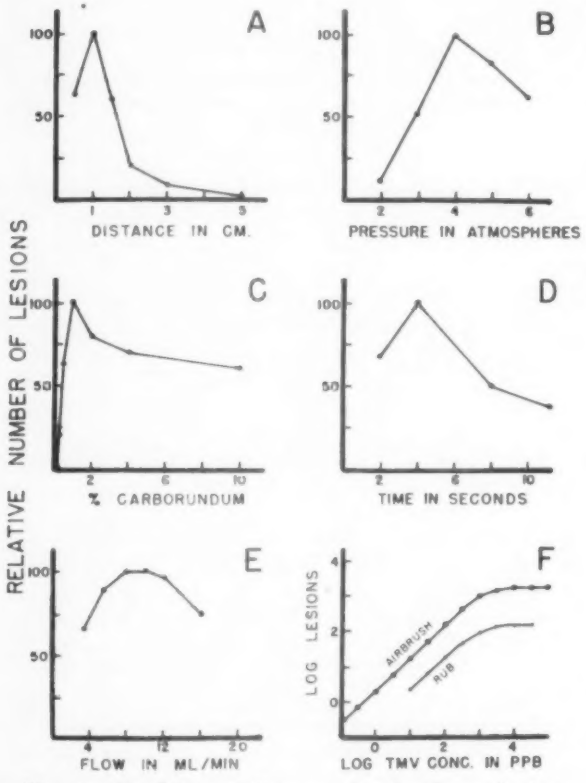

Fig. 1. **A)** Effect of distance of airbrush nozzle from cotyledon surface on relative number of TMV lesions per cotyledon. **B)** Effect of air pressure on relative number of TMV lesions per cotyledon. **C)** Effect of carborundum concentration in the inoculum on relative number of TMV lesions per cotyledon. **D)** Effect of number of passes (about 1 per second) of the airbrush, parallel to mid-rib, on relative number of TMV lesions per cotyledon. **E)** Effect of liquid orifice size on relative number of TMV lesions per cotyledon. **F)** Effect of TMV concentration on relative number of lesions per cotyledon. (PPB = parts per billion = mμg/ml).

The optimum conditions for TMV infection of cucumber cotyledons were: 1) 1-cm distance between airbrush nozzle and leaf surface, 2) 4 atmospheres air pressure, 3) inoculum delivery rate of 10 ml/min., 4) 1% by weight of 600-mesh carborundum as abrasive, and 5) inoculation time of about 4 seconds to cover cotyledon completely.

Results.—The effect of varying the inoculum concentration of TMV on the number of lesions per cotyledon is shown in Fig. 1-F. It will be noted that, with the airbrush, sensitivity is increased about 10-fold over the conventional rubbing inoculation with a cloth pad. There is a concomitant 10-fold increase in maximum numbers of lesions produced per cotyledon. The lesion curve fits precisely the formula previously formulated (2), where $Y = (pVaXsfC)\ (1+pVaXL)^{-1}$. The difference in the lesion curves in the two inoculation methods lies in the "sf" term, which is a term for inoculation efficiency, where "s" represents the fraction of susceptible epidermal cells, and "f" represents the fraction of epidermal cells injured properly. The data (Fig. 1-F) indicate that inoculation efficiency by the rubbing method was 1.75% (sf = 0.0175), whereas efficiency by the airbrush method was 19.6% (sf = 0.196), representing an 11-fold increase in inoculation efficiency. In other experiments, inoculations made with the airbrush gave inoculation efficiencies varying from 20 to 30%. The highest efficiency in a single test was 40.3%.

Variability in lesion counts between cotyledons is less with airbrush inoculations. The coefficient of variation was about 33% for airbrush inoculations, vs. 57% for rub-inoculations. Thus, for equal precision, only ⅓ as many replicates would be required with airbrush inoculations.

Although the airbrush markedly improves inoculation efficiency, variation in cell susceptibility remains an important factor. Moreover, the apparent influence of environmental factors on cell susceptibility appears to be affected by the inoculation method. Diurnal variation in susceptibility is less when the airbrush is used (100% with rub-inoculation vs. 20% with airbrush). Shading rub-inoculated test plants before inoculations reduced susceptibility about 90% (2). Shaded plants inoculated with the airbrush showed 30% more lesions than unshaded controls. However, when test plants were grown at 32°C before inoculation instead of the usual 21°C, susceptibility was reduced about 90%, close to that of rub-inoculated plants. Likewise, when test plants were chilled for 2 hours before inoculation at 2°C, susceptibility was reduced about 80%. Thus the apparent effect of light on cell susceptibility was influenced by the inoculation method, but that of temperature was not.

The effectiveness of the airbrush in TMV inoculations prompted trials with less stable viruses that occur in lower titer. Duplicate lots of inoculum prepared from cucumber cotyledons infected with stone-fruit ring spot virus yielded 16 times as many starch

lesions on cucumber cotyledons with the airbrush than with rub-inoculation. Serial dilutions of this inoculum gave an end-point with the airbrush at a 10-fold greater dilution than with rub-inoculation. Similarly, when the inoculum of this virus was prepared directly from sour cherry petals, starch lesions produced on cucumber cotyledons were 26 times as great when inoculated by airbrush. An unidentified virus was transferred from raspberry to cucumber, and airbrush transfers of this virus from cucumber to cucumber gave 15 times as many starch lesions in cucumber as transfers by conventional inoculations. Thus, when cucumber is the test plant, airbrush inoculations can probably be achieved with virus titers that are 1/10 to 1/20 those of conventional inoculation methods. This may be of considerable importance when working with viruses of low titer.

DISCUSSION.—A 10- to 20-fold increase in efficiency with airbrush inoculations is apparently achieved by increasing the number of epidermal cells that are properly injured, and by reducing the effect of excessive injury on cell susceptibility. Excessive injury to the test plant is virtually eliminated, as evidenced by visual examination of airbrush-inoculated cotyledons. Still greater efficiency might be achieved if the carborundum were eliminated and the water droplets in the spray reduced to about 1 μ in diameter. The water droplets themselves might serve as an abrasive if applied with sufficient velocity. This was not possible with the equipment used. Microscopic measurement of ink droplets applied with the airbrush indicated a mean diameter of 29.3 μ, ranging from 4 to 96 μ. The area covered by the average droplet was thus 78% of the area of the average cucumber epidermal cell (2). To obtain spray droplets of 1 μ diameter and with sufficient velocity to penetrate an epidermal cell would probably require specially designed equipment.

Previous studies have indicated that one carborundum particle per epidermal cell gives the injury optimum for infection (2). With rub-inoculation, this is achieved with 5% by weight of 600-mesh carborundum in the inoculum. Since 1% carborundum was optimum in airbrush inoculations, it was of interest to calculate the mechanics involved. Since 600-mesh carborundum, with a mean diameter of 7.8 μ, contains 6.64×10^8 particles per gram, there will be an average of 1 carborundum particle per 11.6 droplets of inoculum when 1% carborundum is applied with the airbrush. This figure was confirmed by spraying 1% carborundum in a solution of fluorescein on a glass slide and microscopically counting the number of dried droplets containing carborundum particles.

It is possible to approximate the average size of the infection site if one assumes there is but 1 infection site per cell. The dilution curve indicates that lesions are produced in maximum numbers at about 10 μg TMV per ml. With randomly distributed TMV, each TMV particle at this concentration occupies a volume of 8.2 μ^3 of inoculum. If one visualizes the infection site to be the drop of cytoplasm that momentarily protrudes through a wound (2), then the chances of capture of a TMV particle should be proportional to the volume of inoculum momentarily displaced by the infection site. Since there is 1 carborundum particle per 11.6 droplets of inoculum, and each droplet covers 78% of the epidermal cell area, it may be expected that the odds of a capture are 9:1 at any given spot on the cell. When each TMV particle occupies a volume of 8.2 μ^3 of inoculum, the odds of capture must be 1:1, for maximum numbers of lesions are formed at this concentration. Therefore, the volume of the infection site must be $8.2\ \mu^3/9 = 0.91\ \mu^3$. This figure is quite close to that of 0.87 μ^3, calculated previously from rub-inoculated cotyledons (2).

The apparent effect of light on host susceptibility depends on the inoculation method, but the effect of temperature is independent. Therefore, these environmental factors for susceptibility must influence different mechanisms. If the infection site is the drop of cytoplasm that momentarily extrudes through a wound of optimum size, it is likely that chilling plants before inoculation reduces susceptibility by inhibiting such extrusion. This may also be the case when plants are grown at 32°C before inoculation. The effects of light, however, must be more indirect, and the mechanism involved must be affected by the amount of inoculation injury. Virus inhibitors have been reported in cucumber tissue (4, 5, 6); and, in our work, when TMV was applied in a buffered cucumber leaf extract, about 50% fewer lesions were produced than when TMV was applied in the buffer alone. The concentration of such inhibitors may be influenced by light, and they may reduce infection when cells are excessively injured during inoculation. Other factors, such as thickness of epidermal cell wall and amount of cutin, may also be involved.

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WOOD DECAY OF THE GRAPEVINE AND ITS RELATIONSHIP WITH BLACK MEASLES DISEASE [1]

Luigi Chiarappa [2]

SUMMARY

The amount of wood decay in trunks and branches of grapevines in California was correlated with incidence of black measles. From symptomatology, etiology, and control, black measles was concluded to indicate the same pathological condition of the vine described in Europe as "esca," or "apoplexy." Several different genera of fungi were isolated from decayed wood tissues. Of these, only *Fomes igniarius* and a *Cephalosporium* sp. were found to be prime pathogens. The morphological and cultural characteristics of these two organisms and of *Stereum hirsutum* were investigated. From these studies and parallel pathogenicity tests it was found that the wood decay was erroneously ascribed in France to *S. hirsutum* or to a similar species, *S. necator*. The validity of the latter species is questioned, and its rejection is proposed.

INTRODUCTION.—Rotting of the heart and sapwood of the trunks and arms of grapevines appears to harm production and vineyard longevity. Even though such internal decay has been known for many years, the problem has received little attention, probably because economic losses from wood rot have never been experimentally demonstrated. Such a demonstration would be extremely difficult, because of the nature of the disease and the interference of many extraneous factors. Much more consideration has been given to the association of trunk rot with the pathological condition of the vine known as black measles, Spanish measles, or esca. The symptomatology of this disease is more apparent, and the impact of economic losses is directly measurable in reduced yields. In various vineyards of the San Joaquin Valley of California, annual losses from black measles are in the range of 4–25%.

This study was conducted to obtain information on the organisms causing wood decay of the grapevine and to determine their relationship with black measles.

REVIEW OF THE LITERATURE.—Some attention was given to wood rot of the vine in France around the turn of the century. The investigators were primarily concerned with a disease known variously as folletage, apoplexy, and esca, a pathological condition manifested in a sudden wilting of bearing vines or vine parts in midsummer, followed by death. A milder form of the disease was characterized by the appearance within interveinal leaf tissues of chlorotic, yellowish islands, which later became necrotic. Vines affected by the mild form sometimes remained alive for several years. The cause of this disease is not known (5).

As early as 1873 de Rolland (36) observed that the wood of folletage-diseased vines was almost entirely decayed, and that cryptogamic vegetation was abundant in rotted tissues. Association of internal wood decay with the disease was confirmed by Ravaz (30), who, in histological studies, observed that fungus mycelium was always associated with diseased tissues of apoplexed vines, indicating the parasitic nature of the disorder. Sporophores found by Ravaz (31) on apoplexed grapevines were tentatively identified as belonging to the fungus *Fomes igniarius* (L. ex Fr.) Kickx. Pathogenicity tests conducted with this organism were unsuccessful (31). In 1909, Vinet (39) recorded for the first time the presence of *Stereum hirsutum* Willd. ex Fr. on vines showing esca symptoms in Anjou (France). Microscopic studies of this organism within host tissues were made by Rives, in 1921 (33) and again in 1926 (34). In 1922, Gard (15) reported resupinate forms of *F. igniarius* from grapevines with internal trunk decay in the southwest of France. These forms were considered morphologically different from isolates of the same organisms obtained from oak, acacia, and various fruit trees. The varietal name *F. igniarius* (L.) Fr. var. *viticidus* was then proposed by Gard. Patouillard (28), however, on re-examining some of the sporophores collected by Gard, observed none of the reported morphological differences, and therefore discounted the validity of the new variety. In 1926, Viala (38), in an extensive monograph on the history, distribution, symptomatology, and control of esca, reported some of the results of his studies of trunk rot and its causal organism. He admitted that *F. igniarius* could attack the vine, but believed such attacks were exceptional. He mentioned that he had never been able to obtain cultures from fruiting bodies of *F. igniarius* collected on grapevine trunks. Viala believed that a basidiomycete in the family *Thelephoraceae* was the main cause of the decay. This fungus was considered to be either a botanical variety of *S. hirsutum* or an entirely new species, called *S. necator* Viala. Separation of this species was based on its parasitic physiologic specialization on the vine and on a few morphologic characters. Sporophores of *S. hirsutum* from oak were used for morphologic comparisons with the grape isolates. Rives (35), also in 1926, reported that the organism isolated by Viala was indistinguishable from *S. hirsutum*, and not necessarily a new species.

No critical studies on wood decay of the grapevine have since been made. Most of the reports in the recent literature deal with the occurrence and control of esca in growing areas throughout the world. *S. neca-*

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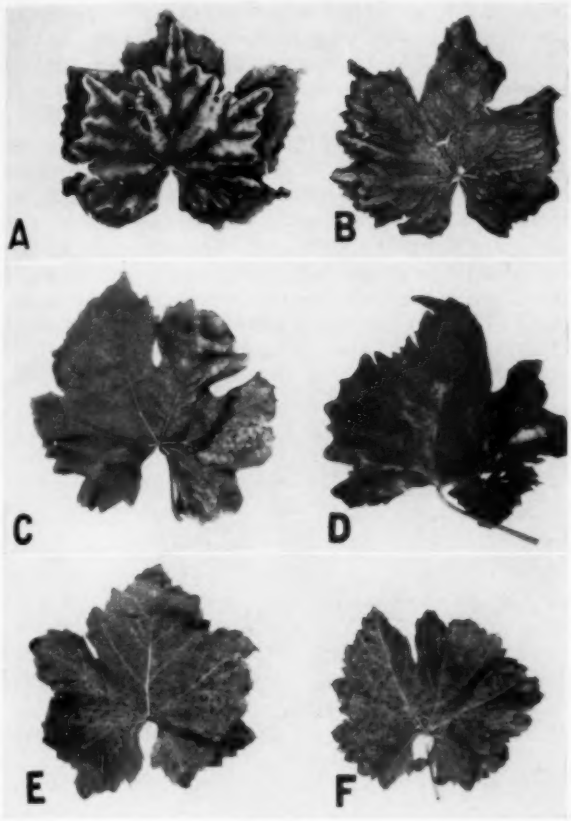

Fig. 1. Various types of leaf symptoms of black measles. **A B)** Interveinal chlorosis and necrosis of Thompson Seedless leaves. **C D)** Clearing, puckering, and distorted growth of leaves of the Thompson Seedless variety. **E F)** Leaves of the variety Kandahar showing numerous small, necrotic areas that give the leaf a metallic-bronze cast.

tor, *S. hirsutum*, and, in a few cases, *F. igniarius* are referred to as the causal organisms.

The only report of heart rot of grapevines in California is by Bonnet (4), who observed some correlation between occurrence of trunk rot and black measles symptoms without making any attempt to identify the organisms involved in the decay. Bonnet suggested that the common form of black measles was identical, or closely related, to the disease known in European literature as apoplexy, or esca. Bioletti (3) compared black measles to water berry and other vine troubles related to overproduction.

SYMPTOMATOLOGY OF BLACK MEASLES. — Black measles in California appears in severe and mild forms. The severe form is frequently encountered during the earliest part of the growing season (May–June). It is characterized by a sudden apical dieback of one or more shoots, accompanied by leaf dropping and the shriveling, bronzing, and drying of fruit clusters. Leaves that do not drop show various patterns of necrosis and bronzing. New growth frequently occurs from the axillary buds of the proximal, unaffected portion of the shoot, soon masking the early symptoms. In severe cases diseased shoots may die entirely.

The mild form of black measles is more common in California vineyards. Symptoms may develop throughout the growing season, with highest frequency in July and August. Leaf symptoms are extremely variable. The most typical consist of small chlorotic areas, which develop between the primary veins and gradually enlarge, becoming necrotic. In advanced stages of mild black measles, the only green tissue left borders the main veins (Fig. 1-A,B). In colored varieties of grapes, a dark red margin surrounds the interveinal necrotic zones. Less common leaf symptoms are a clearing of the veins and interveinal tissues, puckering, and a general glistening confined to limited areas of the leaf blade. These confined areas later become necrotic, with the rest of the leaf remaining normal. Young leaves thus affected become considerably distorted as they grow (Fig. 1-C,D). Some leaves, however, show no chlorosis or clearing; their normal green is merely replaced by a scattering of numerous metallic-bronze areas over the entire leaf surface (Fig. 1-E,F).

Fruit symptoms consist of dark, purple spots scattered over the epidermis of the berries (Fig. 2), appearing any time between fruit set and ripening. The entire cluster may be affected, or only one side, or just a few berries. Severely affected berries sometimes crack and dry on the vine, but may remain fully turgid until maturity.

Spotted fruit of certain varieties, such as Emperor, Red Malaga, and Thompson Seedless, have a slightly pungent, aromatic, and very characteristic flavor.

Fruit symptoms may be present without leaf symptoms, and vice versa. One or more shoots or canes may be diseased, or the entire vine. Symptoms may be present one year, and absent the next. Only a very few vines show symptoms for several years in succession (19).

Diseased vines are often uniformly distributed in the vineyard. Vines 15 years old or older are most severely affected. The disease is known to occur throughout the major growing areas of California.

CORRELATION BETWEEN BLACK MEASLES AND WOOD DECAY.—A Red Malaga and a Thompson Seedless vineyard in the Delano area of the San Joaquin Valley were selected for this study. Records of disease occurrence for individual vines over a 6-year period were available at the Department of Plant Pathology at Davis (19). Six Red Malaga and four Thompson Seedless vines with a known history of black measles were selected at random during the summer of 1956. The following winter they were cut at the soil level and brought to Davis. Trunks and lateral branches were sectioned, and the amount of decay was measured. Decayed wood appeared soft and spongy (Fig. 3), and of a light yellowish color typical of the rot produced by lignin-dissolving fungi. The incipient decay, as observed in median, longitudinal, and cross sections, was bordered throughout by a brownish dark zone of irregular width and outline. Fine, irregular brown lines were observed within the spongy tissue. Both heart rot and decay of sapwood were present. Wider decayed areas were found in the upper region

of the trunk, where pruning wounds are present in greatest concentration. In all cases, the decay gradually became narrower toward the base of the trunk. No butt rots were observed.

Sectioned vines were divided into 3 arbitrary categories on the basis of decay in the main trunk: *Severe*—Rot extending from the top to near the crown of the vine and reaching the live sapwood transversely in more than one area. *Medium*—Rot not reaching the crown, and involving live sapwood in only one region. *Light*—Rotted areas confined to limited zones, and not reaching the sapwood at any point.

Randomly selected vines with no history of black measles (Table 1) had only light decay or none. In contrast, vines that showed disease symptoms in any of the years 1951–56 had medium or severe wood rot. Intensity of symptoms in 1956 appeared unrelated to the amount of decay.

Removal of decayed trunks and arms of black-measles-diseased vines resulted in total disappearance of the symptoms. This was determined by cutting 10 vines back to the soil line in the winter of 1956, and 155 vines the following winter. The following spring, one water sprout was allowed to grow from each of the decay-free old crowns and trained onto a stake. None of the 96 vines obtained in this manner showed

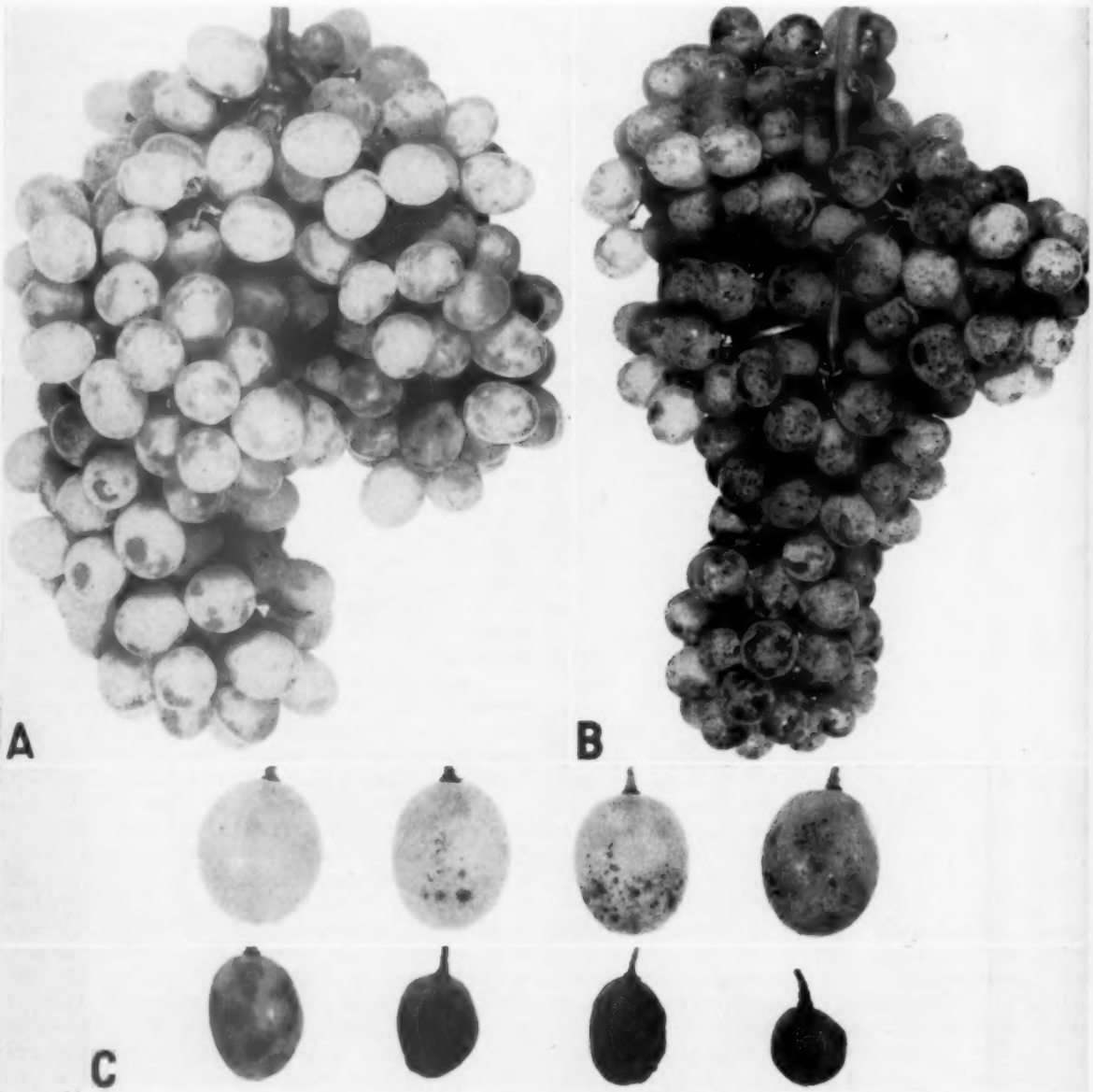

Fig. 2. Fruit symptoms of black measles disease. **A)** Normal fruit cluster of Thompson Seedless at maturity. **B)** Mature cluster of the same variety showing spotting of the berries. **C)** Berries of Thompson Seedless displaying increasingly severe spotting and bronzing. The berry at the upper left is normal, those at the lower right show drying and cracking.

disease symptoms by the end of 1 and 2 growing seasons. All these vines had had black measles symptoms in any of the years 1951–56.

Sodium arsenite as a dormant spray is known to be effective in controlling black measles. For good results, the solution must be applied to the entire surface of the trunk and arms, and must thoroughly wet the old pruning wounds (4, 18, 38). Whether the same effect could be obtained by applying the same material to the soil around the vines or within the decayed areas of the vines themselves is not known from the literature. The effectiveness of these treatments was investigated in 2 separate experiments.

The soil spray application, carried out in a commercial Red Malaga vineyard in February, 1957, consisted of spraying a portion of the soil in the middle row and around the base of the vines so as to imitate a dripping of spray material during commercial applications. The spray concentration, expressed as arsenic trioxide, was the recommended 2.7 lb. of active material per 100 gal. of water, applied at the rate of 350 gal./A. Symptom readings in September, 1957, showed no reduction of black measles.

Eleven Red Malaga and 10 Thompson Seedless vines were used in a parallel test in which 1–4 liters of a solution of sodium arsenite (5 ppm of active material) were injected into the center of rotted areas within the main trunk. The method of injection was similar to that described by Brooks and Moore (6). Injections were made in March, April, and May, 1957. About half of the treated vines developed disease symptoms, in line with expectancy of black measles occurrence in any 2 consecutive years in vines with a long disease history (19). It was concluded that

Fig. 3. Internal trunk rot of a Thompson Seedless vine. **A)** Sectioned trunk exhibiting the widest decayed areas in the "head" region, and no decay at the crown level. Arrows indicate probable points of entry of wood-rotting organisms. **B)** Detail of a sectioned "head," showing the typical white, spongy decay, with zone lines at the margin and within the rotted area. The punk in the section at the right has been removed to reveal depth of decay.

TABLE 1.—*Relation of black measles occurrence to the internal trunk decay of Thompson Seedless and Red Malaga vines*

Variety and vine number	Black measles history (no. of years sequence)	Symptom intensity 1956	Trunk decay index*
Red Malaga			
1	6	Mild	Severe
2	3	Severe	Severe
3	3	Mild	Severe
4	2	Mild	Severe
5	2	Mild	Medium
6	0	None	Light
Thompson Seedless			
7	5	Severe	Severe
8	0	None	Light
9	3	Severe	Medium
10	0	None	None

*Severe—rot extended from the top to near the crown and involved apparent live sapwood; medium—rot did not extend to the crown and involved live sapwood at only one location; light—rot confined to limited areas, mostly heartwood.

arsenic inside the decayed wood was not effective. The efficacy of the chemical therefore appeared to depend on the amount absorbed through pruning wounds or cracks into the live sapwood of trunks and arms. This suggested some neutralizing action of arsenic, either on the activity of wood-rotting fungi or on some toxic metabolites liberated during the decay process.

ISOLATION, MORPHOLOGY, AND PATHOGENICITY OF WOOD-INHABITING FUNGI OF THE GRAPEVINE.—*Materials and methods.*—Material for all isolations was secured from 3 main sources: A Red Malaga and a Thompson Seedless vineyard near Delano (San Joaquin Valley), and a White Pinot vineyard near Rutherford (Napa Valley), all European grape varieties.

Isolations were made by tissue plating in the usual manner from the margins and other areas of typically rotted wood of trunks and branches. Representative samples were always secured from different positions on the vine.

Culture media commonly used were PDA (potato-dextrose agar), Difco bacto-malt agar (23) and Viala's (38) glucose–bean-extract agar modified (tartaric acid increased from 0.5 g to 1.0 g/liter, and agar increased from 15 g to 25 g/liter). Plates were incubated at room temperature unless otherwise specified.

S. hirsutum fruiting bodies were collected in the vineyards or were artificially produced by maintaining grapevine trunks under a discontinuous mist in an unheated greenhouse compartment. Pure cultures of this fungus were obtained from surface-sterilized sporophores and from single spores. The latter were collected on water agar with the method commonly employed to make spore prints in culture media; a modification of the single-spore technique described by Hansen (17) was used for this and other fungi. If no spores were produced, purification was secured by hyphal tip of cultures on water agar.

The keys of Burt (8), Barnett (2), Campbell (9), and Nobles (26) were used for identifications.

Inoculations of living vines in the field, greenhouse, and lath house were made by different techniques, most commonly by drilling, at the base of a spur on one of the branches of cordon-pruned vines, a hole 1 cm in diameter and deep enough to expose the central pith. The hole was then nearly filled with fungus mycelium, plugged with moistened cotton, and sealed with a common cork. Another method of inoculation consisted of forcing a spore or mycelial suspension into the vascular system of dormant vines just before spring growth started. The custom-built hydraulic injector used was similar to that described by Williams (40). Water was used in control plants. The place of inoculation varied with the shape of the vines, but an attempt was made to inoculate the main trunk 25–30 cm below the dividing point of lateral branches. Only bilateral cordon-trained vines of the variety Red Malaga were used for this type of inoculation. From 10 to 100 ml of suspension were used per treatment, the amount depending on the ability of the vine to withstand high pressure.

A third method of inoculation consisted of feeding spore suspensions (distilled water for controls) into the trunk of potted vines by a technique essentially the same as that described by Brooks and Moore (6).

Inoculations of a *Cephalosporium* sp. into green shoots of potted Red Malaga vines were made by hypodermic injection or by the incision method described by Goss and Frink (16). A thin plug of fungus mycelium or 1 ml of a spore suspension was used.

The ability of each isolate to produce decay was determined on blocks and sawdust of sound grape wood under sterile conditions in the laboratory. The healthy wood of a freshly felled seven-year-old vine, variety Kandhahar, was the source of sawdust and blocks. Bark was scraped off and the trunk was sawed into thin transverse sections. Sawdust thus obtained was divided into 10-g lots in 1-pt Mason jars, and 20 ml of distilled water was added with an atomizer. A solution of l-asparagine (2 g/liter, 10 ml per jar) was found effective in speeding up the growth of wood-inhabiting fungi. Jars of moistened sawdust were incubated 36 hours at room temperature, and then fumigated with propylene oxide impregnated into blotter paper, about 4 ml per jar. Fumigation time was 48 hours. Sterile distilled water was added aseptically as needed during the course of the experiment. Five ml of spore or mycelial suspensions were introduced in each jar for inoculation.

Nordin's (27) modification of Koch's third postulate was observed in parallel experiments with fresh wood blocks. Disks of sound wood, 2.5 cm in diameter and 0.2–0.3 cm thick, were thoroughly washed in distilled water and fumigated 48 hours with propylene oxide in closed containers. Four disks were aseptically placed in a sterile Petri dish on a raised glass support and inoculated with uniform fungus-mycelium plugs, 1 plug per disk and 1 organism per plate. All Petri dishes were then enclosed in an inverted glass pipette-

washing jar to reduce interference of laboratory contaminants.

In the description of the macroscopic morphology of fungus isolates the quoted colors were taken from "A Dictionary of Color," by Maerz and Paul (25), or "Color Standards and Color Nomenclature," by Ridgway (32). Common colors, such as white, grayish, brown, etc., were used when no more specific color seemed applicable. The nomenclature describing the texture of mycelial mats was the one suggested by Long and Harsch (24).

Experimental results.—1) Isolations.—Tissue plating of advanced decayed wood yielded species of the genera *Trichoderma, Rhizopus, Mucor, Aspergillus, Penicillium,* and *Cladosporium.* Platings of sound wood in the marginal brown zone almost consistently yielded a species of *Cephalosporium.* Pure cultures of this organism were secured in most cases. The *Cephalosporium* sp., however, was frequently accompanied by a species of *Penicillium.*

Another organism frequently isolated from the same marginal tissues was a basidiomycete tentatively identified, on the basis of cultural characteristics, as *F. igniarius.*

A few isolates of *S. hirsutum* were obtained from a decayed branch of a Red Malaga vine when a portion of the spongy wood bordering the zone line was used for plating.

Although growth of what appeared to be *F. igniarius* was observed on the decayed areas of several trunk sections incubated in a moist chamber, no fruiting bodies or pure cultures were obtained with this technique.

Table 2 lists the fungi obtained in culture and their frequency of isolation.

2) Morphology of isolates.—Circular, slightly raised, slow-growing colonies were produced by the *Cephalosporium* sp. on PDA. Growth in general followed a definite pattern. An initial concentric area, 2.0–5.0 cm in diameter and characterized by abundant sporulation, was formed during the early growth. Once this zone was established, only submerged mycelium was produced, and growth slackened until it ceased altogether.

The color of the fungus colonies varied considerably in some of the strains isolated from grape wood. An eight-day-old isolate from a White Pinot vine was Chicle Sarouk (25), whereas an isolate from a Thompson Seedless was at first cream, and after one week turned to a maize yellow (32). The surface of the medium (PDA) became darker than the aerial mycelium in 2 of the isolates (from White Pinot and Red Malaga), and remained unchanged in the third one (from Thompson Seedless).

Respective optimum temperatures for the Thompson Seedless and White Pinot isolates on PDA were 25° and 27°C. From all the data it appeared that the differences among isolates in optimum temperature were minor at most.

Microscopic structures of all isolates varied but little. Mycelium consisted of abundantly ramified hyaline hyphae, becoming darker in the center of the colony. Width of hyphae varied from 1.0 to 3.0 μ; distance between septa was 9–15 μ. Thickened aerial bundles of brown hyphae, resembling coremia, were observed on oatmeal agar. The conidiophores were simple, unbranched, not swollen at the apex, aseptate, and inserted at right angles. The conidia were hyaline, sessile, elliptical, mostly aseptate with a few uniseptate (1.5 to 2.5%), and produced in heads. Average size was 1.5 to 3.6 μ.

TABLE 2.—*Frequency of isolation and identity of fungi isolated from decayed wood of Vitis vinifera*

Fungi isolated	No. pure cultures obtained	Frequency %
Cephalosporium sp.	99	59.2
Penicillium spp.	26	15.6
F. igniarius	24	14.4
S. hirsutum	8	4.8
Trichoderma sp.	6	3.6
Aspergillus spp.	4	2.4

The sporocarps of *S. hirsutum* were coriaceous, effuso-reflexed, in some cases resupinate, and often gregarious (Fig. 4). They varied in diameter from 0.5 to 3.5 cm. The color of the upper surface was ochraceous buff (32) with the edges turning into a cinnamon brown (32). The lower surface was smoke gray. Sections indicated that the sporocarps were typically 3-layered, with a definite narrow golden zone separating the intermediate area from the lower, hairy layer. No cystidia or gleocystidia were observed. Basidiospores were 5–8 μ long and 2–3 μ wide.

Cultural and microscopic characters of *S. hirsutum* were as follows: growth was moderately rapid with the plates becoming covered in 3 weeks at room temperature; advancing zone was indistinguishable from the remaining mycelium; mat was white, at first sparse, then cottony; hyphae were of 2 main types; hyaline hyphae did not stain with phloxine, rarely branched, 1.0–3.7 μ up to 6.0–9.0 μ wide, frequently septate, hyphae staining with phloxine were unbranched, of even diameter, 3.0–5.0 μ wide, with rare septa. No special microscopic structures were observed. There was no odor, and the mycelium turned light yellow with KOH.

No sporocarps were observed in *F. igniarius.*

Cultural characters of *F. igniarius* were as follows: slow growth on standard malt medium; plates incubated at room temperature completely covered in 5 weeks; optimum temperature, checked at about 3° intervals from 12° to 33°C on PDA, was 27°C; growth was greatly inhibited but not halted at 33°C; a trace of growth occurred at 12°C; reaction on gallic medium (26) was very strong, with complete growth inhibition; advancing zone remained white, smooth, appressed, with no indentations; mycelial mat on malt agar and in complete darkness was at first white (1–2 weeks), then gradually turned to Naples yellow and chamois (32); brown, translucent droplets formed mostly in proximity to the advancing margin; texture of the mat was felty, compact; reverse was unchanged to the end of 4 weeks, then showed a few brown lines; there was no odor; mycelium turned brown with KOH.

The gross morphology of various cultures showed considerable differences on Dion's (14) liquid medium incubated at room temperature in the dark. Some colonies produced a profuse, chamois-colored (32), felty mycelium; while others showed flattened mats of yellow to Dresden brown (32). Morphologic variability of *F. igniarius* in culture has been reported by various authors (20, 37).

Microscopic characters were as follows: hyphae of 2 main types; hyaline hyphae in the advancing zone or in young, white colonies were frequently branched, vacuolated, of uneven diameter (1.0–3.0 μ up to 4.5–6.0 μ); septa were rare and inconspicuous, with no clamp connections; brown hyphae were unbranched, straight, uniform in diameter (1.5–3.0 μ), with no septa; no cuticular cells were observed; no special microscopic structures or chlamydospores were present.

Fig. 5 shows the gross morphology of 3-week-old cultures of *S. hirsutum* and *F. igniarius*.

3) Pathogenicity of isolates.—All 10 of the potted vines inoculated with the *Cephalosporium* sp. in the green shoots, by slit or hypodermic, remained healthy. A small, elongated, necrotic region 2.5–5.0 cm long was observed near the point of inoculation. In all cases this region was walled off by a ring of suberized tissues.

A total of 64 inoculations in the sound wood of living vines were made. Considerable staining of wood resulted from introduction of the *Cephalosporium* sp. into the trunks or branches (Fig. 6). Narrow, dark-brown streaks extended 10–15 cm from the place of inoculation. The brown was darker than the discoloration frequently resulting from injuries. Reisolations from stained areas yielded the same strain that was used for inoculum. Movement of the fungus in host tissues, however, appeared extremely slow. After 6 months (March–September) the *Cephalosporium* sp. could be reisolated only 6 cm away from the locus of inoculation. It seemed that at least part of the darkened tissues were produced well in advance of the fungus mycelium.

The ability of the *Cephalosporium* sp. to stain grape wood was confirmed by a total of 63 inoculations *in vitro* on sawdust and wood blocks.

From 104 inoculations of *S. hirsutum* made in healthy wood of living vines there were no cases of typical white rot. Reisolations from inoculated vines indicated that the fungus could move freely only in dead pith tissues of trunks and arms, not in the sound wood.

The saprophytic nature of this organism was also demonstrated in experiments *in vitro;* no typical decay was visible after 330 days of growth on grapevine sawdust. Hyphae of *S. hirsutum* were observed to penetrate throughout the sawdust, but this mass re-

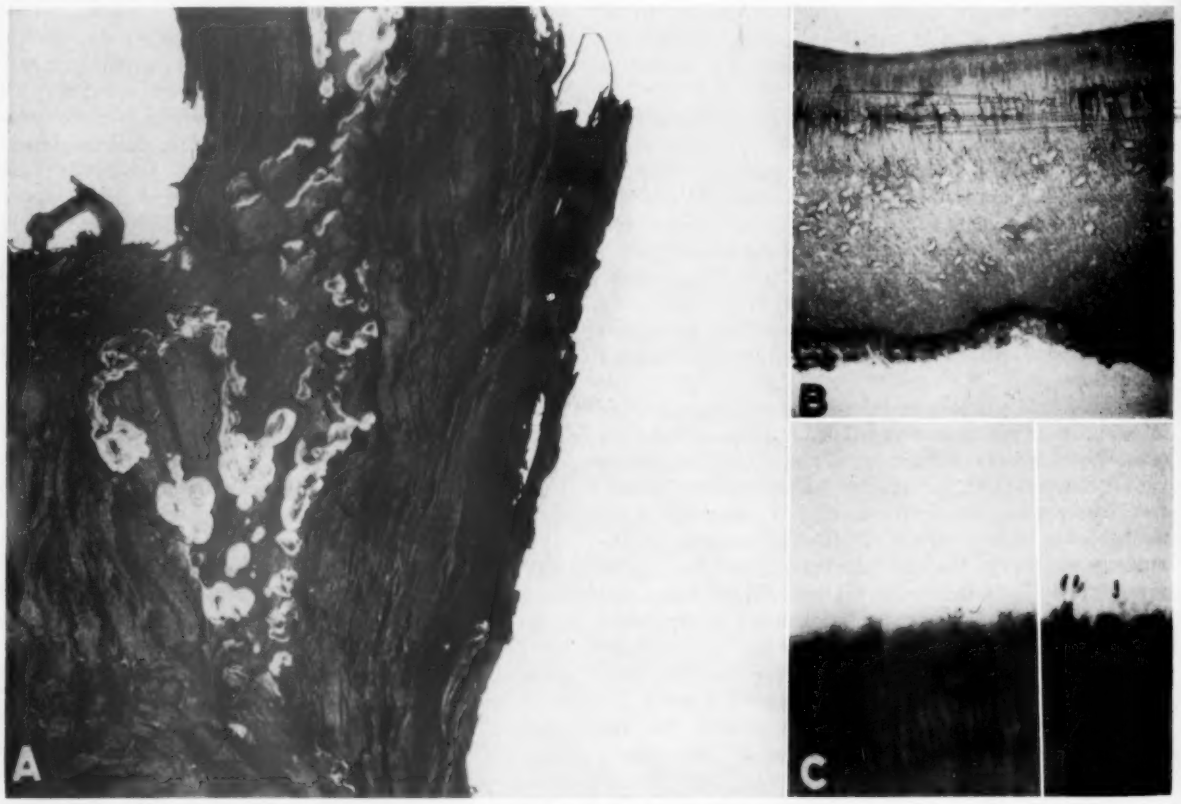

Fig. 4. Fruiting bodies of *S. hirsutum.* **A)** Sporocarps in their natural habitat on a grapevine trunk. **B)** Longitudinal section through a fruiting body (×44). **C)** Detail of the hymenial layer, exhibiting basidiospores on short sterigmata (×300).

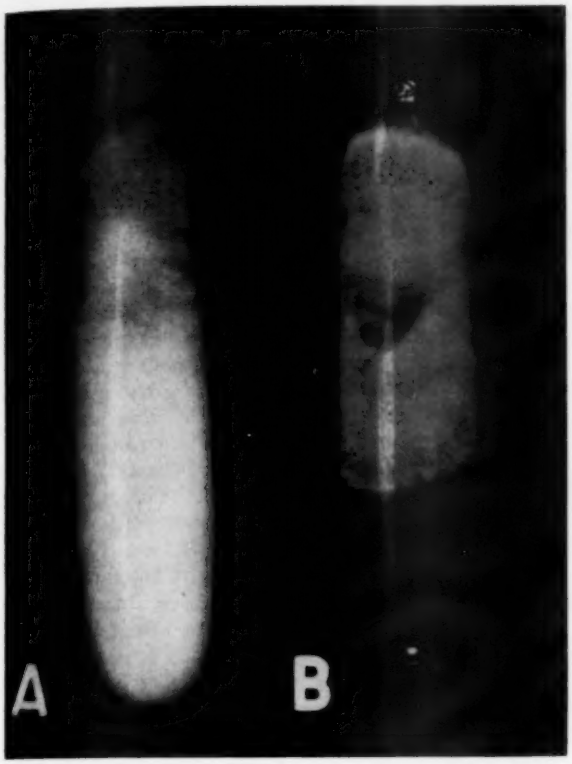

Fig. 5. Three-week-old cultures on PDA of **A)** *S. hirsutum* and **B)** *F. igniarius.*

mained of the same color and consistency as the controls. Wood blocks inoculated with this same fungus were perfectly sound and showed no discoloration after 210 days.

Incipient and typical decay of grapevine wood was obtained when *F. igniarius* was grown both on sawdust and on wood disks. The spongy, yellowish rot produced on sawdust was identical with the decay of standing vines (Fig. 7). Zone lines similar to those described in the literature (21, 37) were visible at the border and within the decayed sawdust. From the phloroglucinol test, most of the lignin in the medium appeared to have been digested. The decay in some jars was not uniformly distributed throughout the sawdust, apparently because the moisture was unevenly distributed. The activity of *F. igniarius* was much slower on wood disks than on sawdust, but the rot 7 months after inoculation had characteristics similar to those in living vines. The decay was most pronounced in disks having a central pith zone, which apparently facilitated establishment and spread of the fungus.

Discussion.—It is recognized that black measles rarely occurs in young vines, and that, in older vineyards, incidence and perhaps severity increases with vine age. Another common observation is that symptoms of black measles have not been observed in old rootstock mother vines, which, year after year, are cut back to the crown to obtain propagating wood. When trunks of vines with a history of black measles were replaced by a water sprout trained from the old stump, new vines were free of symptoms. Vines with extensive internal decay were found to be the ones that had the longest disease record, whereas vines with no heart rot never showed symptoms of black measles. All these observations appear to indicate that the decayed areas are necessary for disease development. More direct evidence should be produced, but it must be pointed out that observations and experimental results obtained so far cannot be explained as mere coincidence. On the contrary, they strongly support cause and effect relation between wood decay and black measles.

Spotting of the berries has never been reported as

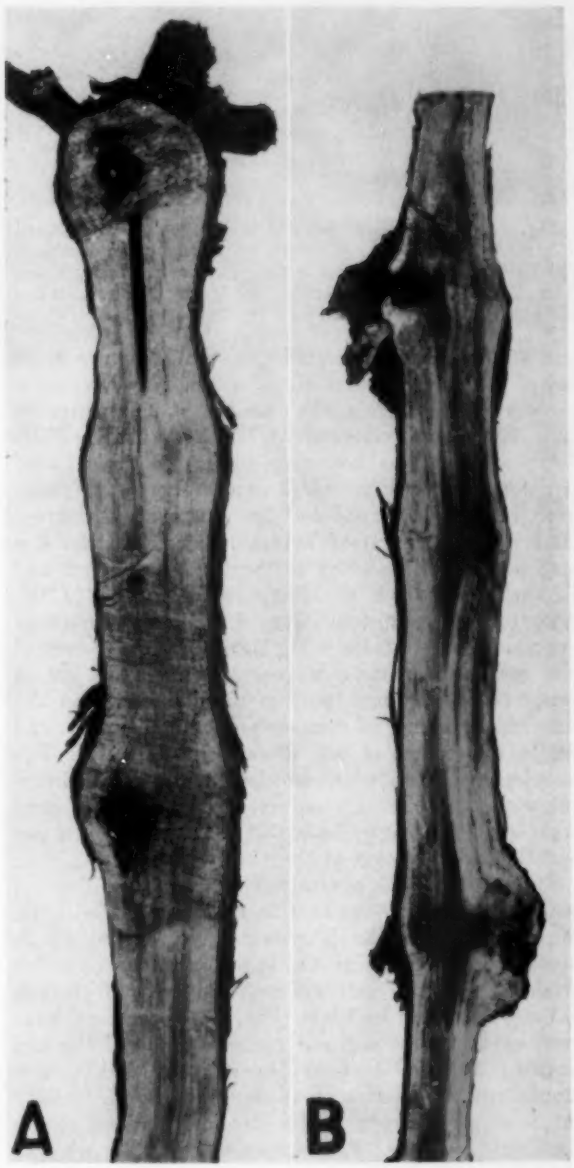

Fig. 6. Sectioned arms of 7-year-old grapevines 6 months after inoculation with a *Cephalosporium* sp. **A)** Control. **B)** Inoculated with mycelial plugs of the fungus at points indicated by arrows.

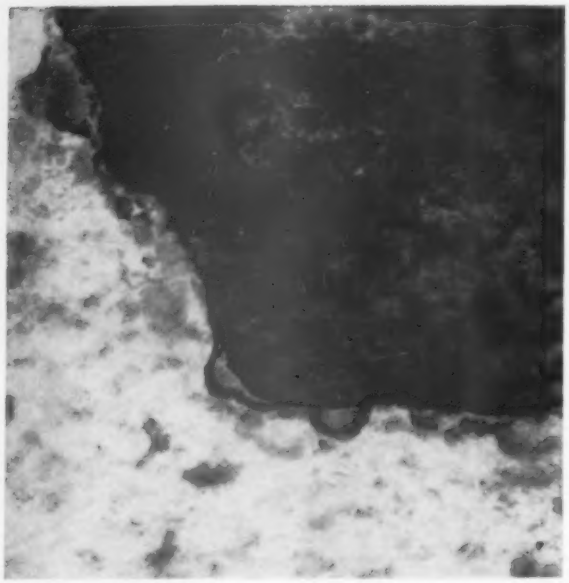

Fig. 7. Typical wood decay of the grapevine, reproduced *in vitro* by growing *F. igniarius* on sterile grape sawdust. The lighter area is identical to the punky rot of living vines. Note the zone lines bordering the decay. Photo taken through the bottom of a Mason jar.

one of the disease symptoms in the literature from any of the growing areas of the world. However, some photographic evidence was obtained of its occurrence in a 50-year-old vineyard of South Australia (12). Berry spotting was accompanied by the typical leaf symptoms of black measles. Similar berry symptoms were recently observed by the writer at Maccarese (Italy) on a Muscat of Terracina vine, and the disease was again observed in the field near Bari and Taranto (Italy) on the Regina variety and in the laboratory of Dr. Antonio Graniti, Istituto di Patologia Vegetale dell' Università di Bari. All symptoms of esca and black measles appear to coincide. Time of symptom development, pattern of distribution in the vineyard, incidence of symptoms as related to age, and finally, the control of both diseases are identical. The correlation of both esca and black measles with internal trunk decay, and, possibly, with the same causal organism, leaves little doubt that they indicate just one pathological condition of the vine.

The results of the present studies on the etiology of wood decay seem somewhat in disagreement with results reported in the literature. Sporophores of *S. hirsutum* collected from California vines were similar in size, color, and external and internal morphology to those described by Viala (38). No agreement, however, exists on the cultural characteristics of the two isolates. On the contrary, microscopic and macroscopic cultural characters, as described by Viala (38) for *S. necator*, appear to be identical to those of the California isolate of *F. igniarius*. Viala (38) was not able to germinate basidiospores of *S. necator*, but instead used tissue cultures from decayed wood throughout his work. There is, therefore, reason to believe that he actually recovered and studied the mycelium of *F. igniarius*. The constant presence of *S. hirsutum* sporophores on diseased vines may then account for his erroneous association of the latter fungus with the decay.

The results of the pathogenicity tests clearly indicate that *S. hirsutum* is unable to produce the typical decay of the vine, either *in vivo* or *in vitro*. The limited number of isolations of this organism from decayed tissues, and its presence always in areas far away from the advanced margin of the rot, seem to suggest a saprophytic nature for this organism. In light of these findings, and because no parasitic specialization or morphologic differences appear to distinguish the grapevine isolate of *S. hirsutum*, it is proposed that the species *S. necator* Viala be rejected.

The soft and spongy decay of grapevine wood appears to be similar to the typical white rot described in the literature (21, 37) as caused by *F. igniarius*. This organism is considered one of the most destructive wood-rotting fungi, attacking both the heartwood and the sapwood of a great variety of trees (21). No reports were found in the literature of *F. igniarius* on grapevine in the United States. Whether isolates from other trees are able to produce the same decay on grapevine remains to be ascertained.

The consistency of recovery of a *Cephalosporium* species from the darkened areas bordering the decayed wood poses the question of the role of this organism in the disease complex. That the *Cephalosporium* sp. does not behave simply as a secondary organism was shown by its ability to move freely into healthy wood and to cause definite staining. Our observations in this regard corroborate the results of Petri (29), who was able to produce brown discolored stripes by inoculating *Chephalosporium* isolates into healthy grapevine wood. The same author reported observations of fungus mycelium in the darkened vessels.

It is interesting to note that numerous vascular disorders have been recently ascribed to various *Cephalosporium* spp. (1, 7), and that these were not limited to herbaceous plants, but included hosts such as the American persimmon (13) and the white elm (16). Some authors also suggested that toxic metabolites are liberated within the plant by this fungus (10, 11, 22). It thus appears probable that the *Cephalosporium* sp. may have some function in the decay process, and eventually in the black measles syndrome.

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OPEN CALYX TUBES AS A FACTOR CONTRIBUTING TO CARPEL DISCOLORATION AND DECAY OF APPLES [1]

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SUMMARY

Discolored and moldy carpels occur sporadically in Wagener and Red Delicious apples. Calyx tubes often extend into the carpels of affected apples of both varieties. *Fusarium* sp., *Botrytis* sp., *Alternaria* sp. and *Aspergillus niger* isolated from moldy carpels produced discoloration when injected into normal carpels. Mycelium was also found in the calyx tubes of immature apples. Many spray materials, possible fungal metabolic products, and other compounds, especially those with wetting properties, injured carpels when injected into them. A wetting agent solution entered a carpel in 20 minutes through an opening less than 25 μ in diameter. Ten of 26 varieties of apples examined either had tubes open to carpels or had a deep calyx sinus or pit, characteristic of susceptible varieties. Rupture of the calyx tissues by abnormally fast growth following heavy rains which occur after a dry period is suggested as contributing to the condition.

In 1954, many of the Wagener apples from an orchard in south-central Connecticut had brown carpels that were often corky or moldy. The entire crop was used for cider because the injured apples could not be distinguished without cutting them open. The grower reported that the condition was restricted to Wagener apples and had occurred sporadically over many years. This information suggested as a contributing factor a varietal characteristic whose expression was dependent upon irregularly recurring environmental conditions.

Carpel rots or "moldy core" (5) or similar conditions have been reported by others (1, 2, 4, 5, 6, 7, 11, 12), and often were associated with an opening into the carpels from the calyx area, a condition called "open core" by Harrison (6). Fungi isolated from rotted carpels have included: an *Alternaria* sp. (1, 5, 6, 7, 8, 10), a *Fusarium* sp. (1, 6), a *Rhizopus* sp. (1), a *Pleospora* sp. (1), and *Physalospora obtusa* (Schw.) Cke. (10). Harrison (6) noted the suggestion of Carne, Pittman, and Elliott (3) that open calyx tubes and related carpel faults were associated with a light crop of fruit and a rapid and irregular growth of the fruit. Others (2, 4) suggested that faulty or inadequate pollination was a cause. However, Harrison (6) was unable to correlate seed content with moldy core and suggested that pollination was important only as it affected fruit set, which was in turn related to moldy core. Brittain and Blair (2) suggested that the triploid variety, Gravenstein, was more susceptible than diploid varieties to the condition, but found some in diploid varieties. Carpenter (5) suggested that the open calyx tubes were a result of failure of the style bases to close completely. Harrison (6) in a study of the morphology of the calyx and carpels of Wagener apples, could find no openings in very young apples. He found small openings near the carpels of apples 1.5 cm in diameter. He found openings in some old styles of mature Wagener apples, but these openings failed to extend to the exterior of the apples.

EXPERIMENTAL.—Some of the corky carpel tissues resembled the corkiness produced in beets by a boron deficiency. Therefore, samples taken from severely discolored carpels, from slightly discolored carpels, and from apparently normal carpels were analyzed spectrographically by W. M. Mathis, of the Analytical Chemistry Department of the Connecticut Agricultural Experiment Station. No differences in boron content were found among these samples.

Fungi isolated from the discolored carpels of Wagener apples in the present study included species of *Coniothyrium*, *Alternaria*, *Fusarium*, *Penicillium*, *Botrytis*, and *Neurospora*, as well as *Aspergillus niger* Michele. Only the *Alternaria* sp., which was much like *A. tenuis*, was found in carpels of Wagener apples from one orchard; and these apples showed only slightly discolored carpels. To determine the importance of these fungi, 0.2 ml of a spore or mycelial suspension of these isolates was injected with a sterile syringe into carpels of harvested McIntosh apples. After incubation at room temperature for 1 week, the apples were cut longitudinally through the carpels and examined.

The *Penicillium* sp., *Botrytis* sp., *Alternaria* sp., and *A. niger* fruited freely in the carpels. *Fusarium* sp. or *Botrytis* sp. severely discolored and decayed the carpels. *A. niger* and *Alternaria* sp. produced some browning if the carpel walls were cracked at time of inoculation. The other fungi and a culture of bacteria obtained from discolored tissues produced very slight or no browning.

The discoloration might have arisen from fungal metabolic products. Some possible metabolic products were injected into carpels at a concentration of 0.1%. Lactic acid, tartaric acid, oxalic acid, acetic acid, and a pectic enzyme preparation caused severe injury. Ascorbic acid, L-cysteine hydrochloride, paraminobenzoic acid, benzoic acid, nicotinic acid, L-tyrosine, indoleacetic acid, citric acid, and L-malic acid caused very slight to no injury. A mycelial extract of the *Botrytis* sp. caused rather severe browning of the carpels but caused no injury when boiled for 1 hour.

Many of the Wagener apples had open calyx tubes. Could spray materials injure after entering the carpels through calyx openings? To answer this question, spray materials at a concentration of 0.1% were in-

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jected into the carpels of McIntosh apples. After a week's incubation at room temperature the apples were split and examined. Tween 20 (polyoxyethylene sorbitan fatty acid esters), glyodin (2-heptadecyl-2-imidazoline acetate), and an organic mercurial fungicide, *N*-(ethylmercuri)-*p*-toluenesulfonanilide, caused severe browning. Ferbam (ferric dimethyldithiocarbamate), captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide), zineb (zinc ethylenebis [dithiocarbamate]), and cupric sulfate caused slight or no injury. Methoxychlor (1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane), malathion (*S*-(1,2-dicarbethoxyethyl)-*0,0*-dimethyl phosphorodithioate), and nicotine sulfate caused slight to moderate injury. TDE (2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane) caused none. Other materials that might be in pesticides or spray water were tested. Sodium hydroxide caused severe browning. Magnesium sulfate, a soluble fertilizer, and hydrogen peroxide caused slight to no injury. Distilled water injections or needle punctures did not produce discoloration.

Acetic acid and Tween 20 were tested further at concentrations below the 0.1% used in the first trial. Both materials still caused severe injury at 0.01%.

To determine if spray materials could enter the carpels through open calyx tubes, punctures were made with a fine needle, about 25 μ in diameter, through the calyx tube into the carpels of McIntosh apples. Examination of many apples of this variety had failed to find any naturally open calyx tubes so the opening made with the needle would be the only one present. Five-tenths ml of a 0.1% solution of a wetting agent, Tween 20, containing 0.01% acid fuchsin as an indicator dye, was placed in the calyx basin. After various intervals, the dye solution was rinsed off the surface and the apples were split longitudinally for examination. The Tween-20–acid-fuchsin mixture entered the carpels within 20 minutes.

Because of the apparent importance of open calyx tubes to the incidence of carpel rots, it was of interest to know which varieties showed the weakness. Eight harvested apples from each of 26 varieties of apples were split longitudinally through the calyx for examination of their calyx and carpel morphology. Wagener, Delicious, Red Delicious, Golden Delicious, Idared, Davey, Macoun, Gravenstein, and Russett apples either had calyx tubes open to the carpels or had deep calyx tubes. Edgewood, Grimes Golden, Fall Pippin, Opalescent, and Winter Banana apples had average or variable ones. Those apples with shallow calyx tubes or with much tissue between the carpels and the calyx tube were McIntosh, Cortland, Baldwin, Esopus Spitzenberg, Jonathan, Rome Beauty, Red Rome, Roxbury Russett, Ruby, Stayman Winesap, Tompkin's King, Winesap, and Rhode Island Greening apples.

Fig. 1. Calyx structure of 3 apple varieties found with moldy carpels. **A)** Red Delicious apple with calyx tube extending into carpel. **B)** Red Delicious apple with carpel decayed by *Fusarium* sp. which entered by open calyx tube. **C)** Wagener apple with deep calyx tube extending into carpel. **D)** Golden Delicious apple with calyx tube open into carpel.

In 1955 an attempt was made to follow the development of the calyx tube in the Wagener apple. However, the tubes remained closed from the start, despite weather conditions similar to those in the previous year. On the other hand, all of 25 Red Delicious apples examined on August 25, 1955, had mycelium in or just entering the carpels, some through openings too small to detect without sectioning on a microtome; but many of them had visibly open calyx tubes, as seen in Fig. 1-A. In addition, 2 Red Delicious apples showing carpel rots were sent in by orchard men. The causal fungal agents, *Fusarium* sp. in one (Fig. 1-B) and *Physalospora obtusa* in the other, had very plainly entered from open calyx tubes. Thus, the appearance of the condition may differ with area and variety in the same year. Wagener apples were affected in 1954, but not in 1955; the reverse was true for Red Delicious apples. In 1954 Wagener apples in 2 orchards had open calyx tubes (Fig. 1-C), but those in an orchard not too distant from one of these did not have any trouble. Differences in cultural practices or soil types that affect the water supply at the critical stage or stages of development of the apple might be responsible for these differences in occurrence of moldy core among areas or varieties.

Rainfall was nearly normal in 1956, with no prolonged dry spell in June or extra rainfall in August. Wagener and Red Delicious apples were obtained from the orchards where the trouble had occurred before. None of these apples had open calyx tubes. It may be necessary to have a dry spell in June without heavy rains in August, or normal rainfall in June with heavy rainfall in August, to determine whether early dry weather or late excess water are responsible for the trouble, or whether it is a combination of the 2 conditions.

Discussion.—Two facets of the carpel rot problem are: the actual injurious agent, chemical, or organism, and the cause of the open calyx tubes, which permit the entry of this agent. This study has dealt mostly with the first aspect. However, the apple fruit in which the calyx tube is closed is resistant. What caused the open calyx tube? Several writers suggested a connection between open calyx tubes and fast or abundant growth. Open calyx tubes in Connecticut apples in 1954 and 1955 occurred in years having dry weather in late spring and early summer followed by heavy rains in late summer. Deviations from normal rainfall during June, July, and August of 1954 and 1955 were as follows: 1954—June, −2.29 in.; July, −2.62 in.; August, +1.71 in. (2.74 in. on August 30–31); 1955—June, −1.29 in.; July, −1.35 in.; August, +6.84 in. (3.24 in. on August 12 and 4.22 in. on August 18 and 19). Open calyx tubes were found in various apples in 1958 when there was a short dry period in late June and a heavy rain in late August. Lack of water might have prevented closure of the style bases, a condition suggested by Carpenter (5) as a cause of open calyx tubes. Abnormally fast expansion of surrounding parenchyma tissues, which probably resulted from the heavy rains in August, could have caused a separation of the style tubes. Another possible explanation might be the formation of openings in the carpels, such as Harrison found extending part way to the exterior. Abnormal growth during high moisture conditions could have extended such openings to a junction with the calyx tube, forming an opening to the apple's exterior.

It has been shown that fungi might cause some, but not necessarily all, carpel browning. Several fungal metabolic products, such as organic acids or pectic enzymes, injured carpel tissues. It was shown in this study that a fungal extract that had caused injury prior to heating, did not do so after heating. Such heating could destroy materials other than enzymes, but these are especially susceptible to such treatment.

Several organic pesticides caused injury in laboratory trials. The tests with the wetting agent and indicator dye would suggest that pesticides can enter open calyx tubes. Many of these tubes are large enough to permit rapid entry of materials in solution or suspension into the carpels once these materials entered the calyx. Wetting agents in sprays would enhance entrance but also would increase injury to carpels; Tween 20 (a wetting agent) and glyodin (a fungicide with wetting agent properties) caused very severe injury.

Injury by fungicides makes inadvisable the use of certain fungicides to prevent entrance and injury by fungi. Apart from this, open calyx tubes develop close to harvest time, and the use of sprays this late in the season could result in spray residues above the tolerance limit.

Combatting the effect of sporadically occurring unfavorable environmental conditions which are suggested as the cause of open calyx tubes is very difficult. Carpel rots can be largely avoided, however, by planting varieties of apple with shallow calyx tubes since these fruits are much less likely to develop open calyx tubes.

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REPORT OF THE 1959 MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The sixteenth annual meeting of the Potomac Division of the American Phytopathological Society was held February 26 and 27, 1959, in the auditorium of the Plant Industry Station, Beltsville, Maryland. Twenty-seven scientific papers were presented. About 150 people attended this meeting. Following the banquet on the evening of February 26, Dr. Karl Maramorosch showed pictures and discussed problems of viruses and virus diseases on both sides of the "Iron Curtain."

Officers elected to serve during the next year are as follows: *President*, W. D. MCCLELLAN; *Vice-President*, A. B. GROVES; *Secretary-Treasurer*, J. G. MOSEMAN; and *Councilor*, L. O. WEAVER.

John G. Moseman, SECRETARY-TREASURER

Production of lateral roots in soybean varieties resistant and susceptible to Meloidogyne incognita acrita. CRITTENDEN, H. W. Two varieties of root-knot-nematode-susceptible soybeans (Virginia and Adams) and 2 resistant varieties (Laredo and Anderson) were exposed to *Meloidogyne incognita acrita* for periods of 8, 16, and 32 days; these varieties were also grown in uninfested soil for similar periods. When the secondary roots were invaded by the nematode, each of the two susceptible varieties, at 8 and 16 days of age, exhibited a reduction in number of tertiary roots produced (significant at 0.01 level) as compared to number of tertiary roots produced on the same varieties in uninfested soil. Each of the resistant varieties exhibited no change in tertiary root production when grown in infested soil compared to roots produced in uninfested soil. Enlargement of the pericyclic region in numerous areas of secondary roots of susceptible soybean varieties invaded by nematodes has a high positive correlation with the inhibition of tertiary roots in these same varieties. Size of gall caused by *M. incognita acrita* and the number of lateral roots produced in soybean varieties are affected by potassium.

The amino acid and sugar content of cells of Xanthomonas vesicatoria. CROSSAN, D. F. The mucoid growth of *X. vesicatoria* on potato-dextrose agar was harvested and hydrolyzed with $4NH_2SO_4$ for 24 hours at 85°C. Neutralized hydrolysates were reduced to dryness and the samples spotted on chromatographic paper. Papers were developed by descending flow in a mixture of *l*-butanol, pyridine, water, and glacial acetic acid (6:4:3:1). Partridge's reagent and ultraviolet fluorescence were used to identify sugars in comparison with known standards. The following sugars, in descending order of relative abundance, were identified: glucose, mannose, rhamnose, ribose, xylose, arabinose, and galacturonic and glucuronic acid. In determining amino acids, neutralized hydrolysates were reduced in volume to 3 ml, frozen, thawed, filtered to remove salts, spotted on paper, and developed 3 times in a mixture of *l*-butanol, glacial acetic acid, and water (4:1:5). The papers were dried, sprayed with borate buffer at pH 9.5, developed in a second dimension in a mixture of meta-cresol and phenol (1:1), and dried at 40°C. The paper was sprayed with a mixture of 0.2% nin-hydrin in 50 ml ethyl alcohol + 2 ml of glacial acetic acid and then heated at 105°C until dry. Amino acids identified were cystine, lysine, aspartic acid, serine, glycine, glutamic acid, threonine, B-alanine, proline, tyrosine, valine, phenyl-alanine, isoleucine, and leucine.

Cytological and histological effects of Xiphinema diversicaudatum and Meloidogyne hapla on rose roots. DAVIS, R. A. *Xiphinema diversicaudatum* and *Meloidogyne hapla* both stimulate gall formation on rose roots. Since these nematodes differ in their mode of feeding, one being an ectoparasite and the other an endoparasite, cytological and histological effects were compared. Formation of galls on rose roots parasitized by *X. diversicaudatum* was due primarily to a hyperplastic response of the cortical cells. However, giant cells approximately 2–3 times as large as other surrounding cortical cells were formed in association with feeding sites. In some infections, cortical cells lying close to giant cells contained deeply red-staining bodies adhering to or protruding from the cell walls. Meristematic activity in the root tip was retarded. Gall formation due to the feeding of *M. hapla* was characterized by the formation of giant cells, hyperplastic cortical and vascular parenchyma, abnormal xylem, and cessation of root tip growth. Giant cells in differentiated tissues were formed from a group of vascular parenchyma cells, which were first enclosed by a thick cell wall. Walls of the parenchyma cells enclosed by the thick cell wall disintegrated, forming a giant cell. In undifferentiated tissues, giant cells were formed by the dissolution of cell walls, with a subsequent deposition of a thick cell wall.

Etiology of Botryosphaeria ribis on wood and fruit of the Rome apple. EID, R. F., AND J. W. HEUBERGER. Investigations on the etiology of *B. ribis* were intended to obtain information on the life-cycle of this fungus in connection with the occurrence of fruit infection to form a basis for timing fungicide applications for control. This organism attacks apples in Delaware, causing wood cankers and fruit rot. Pycnidia and perithecia containing mature viable spores were found all year on cankers in an infected orchard. Peak of spore production occurred from June 1 to July 15. Optimum climatic conditions for spore germination were 100% relative humidity for several hours and a temperature range of 77–86°F. Susceptibility of apple fruit to *B. ribis* was investigated (a) by artificial inoculation and (b) by bagging of fruit at the petal stage and then uncovering 200 fruit at 15-day intervals throughout the season. Results indicate that fruit is susceptible to infection from the beginning of June until harvest in October. Rotted apples usually fall to the ground, and, when entirely rotted, pycnidia-containing spores are formed under the epidermis. As Rome apples appear to be susceptible to infection in all stages of development, a protective spray schedule must be devised with this in mind and with special emphasis on the period of maximum spore production.

Gold chloride and picric-acid–iodine as in toto stains for free-living and plant-parasitic nematodes. HASBROUCK, EDWARD. Two techniques for *in toto* staining of plant-parasitic and free-living nematodes were developed. In the technique employing gold chloride, nematodes were first washed in distilled water and then transferred to an aqueous solution of 0.1% $AuCl_3$ and 0.1% $HgCl_2$. A gen-

eral light yellow coloration of the specimen indicated complete impregnation. Following a 10-minute wash in distilled water, development of color took place in 1.0% formic acid under a photoflood lamp. Dehydration and mounting in glycerine were accomplished by the method of Baker. Nerve rings and associated cells and gonads differentially stain a bright red. The deposition of a fine black precipitate on the cuticle assisted visual definition of cuticular details. Temporary mounts were routinely stained by .001% iodine and 0.5% concentrated picric acid in formol acetic acid. Killing and fixation of specimens was rapid. Sclerotized and glandular structures were clearly differentiated by the use of this stain.

Lead arsenate as an apple fungicide. HEUBERGER, J. W., AND R. F. EID. In 1958, a block of the Rome variety was used for a large-scale field test. The entire block was sprayed with captan (2 lb.) through the second cover. Beginning at the third cover (June 16) and continuing through the twelfth cover (Sept. 24), the following materials were applied with a speed sprayer on 4-row plots (88 trees): no fungicide, lead arsenate + lime (1½:1½ lb.), captan (2 lb.), captan + lead arsenate (2:1½ lb.), captan + zineb (1:1 lb.), and captan + zineb + lead arsenate (1:1:1½ lb.). Data were obtained on 3 diseases: TERMINAL SCAB—severity index (4.00 = maximum) on October 8, based on 100 terminals on each of 4 trees, was as follows for the treatments, respectively: 3.46, 1.34, 0.19, 0.15, 0.04, and 0.03 (LSD @ 5%P = 0.24). SOOTY BLOTCH —percentage fruit infection on October 7, based on 100 fruit on each of 4 trees, was respectively: 89.5, 14.0, 2.00, 0.00, 0.00, and 0.00. BOTRYOSPHAERIA FRUIT ROT—based on 6 biweekly counts (August 1 to October 7) on 100 fruit on each of 4 trees, the average percentage infection was respectively: 30.6, 16.3, 4.0, 1.5, 0.5, and 0.2 (LSD + 5%P = 2.1); on dropped fruit it was 78.0, 40.1, 18.6, 13.3, 11.6, and 8.7 (LSD @ 5%P = 4.6).

Natural transmission of sweet potato feathery mottle virus by the whitefly Trialeurodes abutilonea, conditioned by climate. HILDEBRAND, E. M. Natural spread of feathery mottle virus (syn. Yellow Dwarf; *Georgia mosaic*) in 1955, 1956, and 1958 averaged only 6% as compared with 28% in 1957, as determined by the "flush of growth" technique. Clean cultivation in 1955 and 1956 was associated with a low natural spread. Omitting cultivation around the planting in 1957 caused the border to be very weedy. Indian mallow (*Abutilon theophrasti*) was present and heavily populated with a whitefly (*Trialeurodes abutilonea*). An extreme drouth in August defoliated the Indian mallow. This was followed by rain and abnormally warm weather in September, and as a result sweet-potato foliage became abundant. Current-season symptoms of the virus such as regularly occur in Kenya, East Africa, and in Georgia were noted only in 1957 on the lush-growing sweet potatoes. Apparently, defoliation of the weed by the drouth shifted the whitefly from the weed to the sweet potato, where it bred, became viruliferous, and caused about 10% current-season foliage symptom. In 1958, when weed growth was not disturbed by drouth and host growth was not enhanced by favorable weather, natural transmission fell back to about 5%—evidence that this whitefly prefers the weed host in Maryland.

Sweet potato ringspot virus associated with internal cork virus. HILDEBRAND, E. M. A ringspot virus apparently free from internal cork was first obtained from a bushel lot of Unit I Porto Rico sweet potato roots (Johnstone # 2) from Georgia in the autumn of 1955. This lot, a part of 30 one-bushel lots of Porto Rico roots from 23 states, was the only clone free of root symptoms after 5 months of warm storage. When grown in an isolated field in 1956, stored, and sliced after 6 and 12 months of warm storage, the roots from all 104 plants were again cork-free, although the symptoms persisted in the foliage. Because all other Unit I Porto Rico material had both root and leaf symptoms, it was concluded that this sweet potato virus complex consisted of at least two distinguishable viruses: the ringspot virus, which apparently is innocuous, and the internal cork virus, the latter causing both root and leaf spot symptoms. This conclusion was verified by using indicator plants, including sweet potato seedlings and morning glories inoculated and infected by aphid, graft, or mechanical transmission.

Mechanical transmission of potato-mottle virus to and from citrus plants. HOLMES, FRANCIS O. Potato-mottle virus was transmitted with difficulty to healthy seedlings of Mediterranean sweet orange, Orlando tangelo, and Meyer lemon by rubbing inoculum into their leaves in the presence of finely powdered carborundum. Subsequently it was recovered regularly and with ease by subinoculation to tobacco from chlorotic lesions that developed in the inoculated leaves, but it was not recovered at all from green areas of the same leaves. The relative ease with which the virus was isolated from locally infected citrus tissues, in conjunction with the difficulty of infecting these tissues originally by mechanical inoculation, seems enlightening. It suggests that earlier failures to transmit mechanically the viruses that occur naturally in citrus plants may be attributable to use of insusceptible or insufficiently susceptible test plants rather than to the presence of an inhibitor in citrus foliage.

Some new information on black knot and brown rot of plums. LEWIS, F. H., AND K. D. HICKEY. Ascospores of *Dibotryon morbosum* were mature when tree growth began and were discharged from then until after bloom. Infections were on new growth of spurs and terminals and new knots appeared in July. Knot removal by pruning was successful only where the cut was low enough to remove all diseased tissue. Knots on large limbs were effectively removed by rough surgery followed by a wound paint of 200 ppm Actidione in water. In spore germination trials, captan, dichlone, and mercury were highly effective against both *Dibotryon* and *Sclerotinia fructicola*. Some materials, especially sulfur, were less effective in the laboratory but gave good black knot control in the field. In field tests on Stanley prune, zineb, Niacide-M, mercury, sulfur, ferbam, captan, and dichlone gave good to fair black knot control, in the order named. Copper plus lime, lime sulfur followed by captan, glyodin plus mercury, and glyodin gave unsatisfactory control. Only captan, dichlone, and mercury gave control of brown rot blossom blight. Captan was safe on the trees, mercury caused leaf injury in the first spray after bloom, and dichlone caused leaf injury in the second spray and fruit injury in the third spray after bloom.

Effects of Meloidogyne incognita acrita and Trichodorus christiei on the nutrient levels of tomato. MAUNG, OHN. To study the nature of mineral deficiency symptoms in plants following nematode attack, and to determine effects of both ecto- and endoparasitic nematodes on plant nutrition, an experiment was carried out with tomato plants inoculated with 2 population levels of *M. incognita acrita* and *T. christiei* and compared with check plants. Sixty days after inoculation, plants were harvested, and tops and roots of each were separately analyzed for N, P, K, Ca, Mg, Na, reducing sugar, non-reducing sugar, and total sugar. Roots of plants having a root-knot index of 26–50 were significantly lower in Na content. No other differences were observed in tops or roots at this infection level. Levels of N, P, and K in roots of plants having an index of 51–75 were found to be significantly higher than those of checks. However, Na was found to be significantly lower in both cases. Plants inoculated with 2500 *T. christiei* showed a tendency to be deficient in over-all nutrient level both in tops and roots, but differences were not significant. There was no difference in nutrient level in plants inoculated with 600 *T. christiei*. No difference was observed in sugar content, regardless of the rate of inoculation in either *M. incognita acrita* or *T. christiei* infected plants.

Greenhouse and laboratory studies on chemical soil drenches to control Phytophthora parasitica var. *nicotianae, the Cause of Black Shank of Tobacco.* MORGAN, O. D. Control of the fungus *Phytophthora parasitica* var. *nicotianae* was obtained in greenhouse tests with 4 Omadine (2-pyridinethione 1-oxide) derivatives, disulfide, zinc, iron, and manganese at 300 and 600 ppm; N521 (Mylone) (3,5-dimethyltetrahydro-1, 3,5, 2*H*-thiadiazine-2-thione) at 300 and 400 ppm; chemical 5400 (alpha-alpha-trithiobis(*N*-dimethylthioformamide) at 600 ppm; and Orthocide 406 (*N*-trichloromethylmercapto-4-cyclohexene-1, 2-diacarboximide) at 300 ppm. Tobacco planted 4 days after treatment of the soil was stunted by concentrations of the chemicals that controlled the fungus. No phytotoxic effects occurred when tobacco was planted 3 weeks after treatment. Laboratory tests indicated that the chemicals inhibited, but did not kill, the fungus after exposure for 4 days. After 7 days' exposure the disulfide, zinc, or manganese derivatives of Omadine at or above 50 ppm and N521 at or above 80 ppm killed the fungus. The iron derivative of Omadine at 200 ppm, chemical 5400 and Orthocide 406 at 400 ppm did not kill the fungus. Spore germination was inhibited by all the fungicides at or above 5 ppm, indicating that spore germination is the weak link in the life cycle. Of the fungicides used, the disulfide and the manganese salt of Omadine and N521 were the most effective against *P. parasitica* var. *nicotianae*.

Investigations on the control of the Rhizoctonia disease of snap beans by green organic soil amendments. PAPAVIZAS, G. C., AND C. B. DAVEY. Five green organic amendments were added singly at the rate of 1% to unsterilized sandy soil naturally infested with *Rhizoctonia solani*. In the greenhouse, initial fallow periods of 0, 10, and 24 days were followed by 3 successive plantings of susceptible Topcrop snap beans. The amendments green corn and oats stimulated the highest numbers of rhizosphere and soil streptomycetes antagonistic to *R. solani in vitro* and reduced the infection index of bean seedlings. These amendments also decreased the prevalence of the pathogen in the planted and unplanted soil as determined by colonization studies. Sudan grass and no amendment (control) resulted in few antagonistic streptomycetes and in the highest infection index, whereas bean and buckwheat amendments were intermediate in these respects. All amendments became less effective in controlling the disease with time after incorporation. No correlation was found between disease severity and the rhizosphere/soil ratios of bacteria, streptomycetes, and fungi.

Susceptibility of various plants to Pratylenchus penetrans as determined by behavior of the nematodes, lesion formation, and root growth. RACE, S. R., AND M. T. HUTCHINSON. *Pratylenchus penetrans* was observed infesting 340 seedlings of 14 plants grown in autoclaved muck soil contained in Petri dishes. Time for the nematodes to locate the root, degree of activity, time for penetration and oviposition, and number of eggs deposited varied considerably. Based on these observations, tomato, lettuce, chrysanthemum, and broccoli were excellent hosts; peach, crown vetch, red clover, and sweet corn were good hosts; strawberry, carrot, turnip, and radish were fair hosts; and blueberry and asparagus were very poor hosts. Blueberry roots were markedly unattractive; and asparagus roots were toxic to the few nematodes that entered. With the exception of strawberry, this laboratory evaluation correlates well with populations of *P. penetrans* found in roots of field-grown plants in New Jersey. Although the nematodes usually penetrated only the mature portions of roots, the tips of feeder roots of broccoli, turnip, radish, and strawberry were commonly entered. In most hosts, lesions of specific colors were produced immediately after penetration. Rootlet growth of tomato, lettuce, red clover, carrot, and crown vetch was reduced to about 50% of normal within 2 weeks of inoculation with 50–100 nematodes. Inoculation of 10 nematodes did not affect rootlet growth of tomato.

Greenhouse studies on sugar beet root rot caused by Aphanomyces cochlioides. SCHNEIDER, C. L. The chronic phase of sugar beet root rot was experimentally induced in the greenhouse with pure single-spore cultures of *Aphanomyces cochlioides* originally isolated from damped-off sugar beet seedlings. Plants were grown singly in 3-in. pots of steamed soil, and when they were 30 days old, a suspension of about 1 million zoospores in 50 ml of water was poured into each pot. Within 6 weeks the plants showed symptoms of the chronic phase of root rot similar to those observed in the field under natural exposure to *A. cochlioides*. Symptoms included severe wilting of the foliage, discoloration and rotting of side roots, decay of the terminal portion of the taproot, and general reduction in size of the taproot. Plants in uninfested control pots remained healthy. Three varieties of sugar beets, which differ in their degree of susceptibility to seedling blight caused by *A. cochlioides*, differed in susceptibility to the chronic phase of the disease, in a similar degree.

Host-parasite relationships of soybean and a root-knot nematode Meloidogyne hapla. SCHILKE, P. J., AND H. W. CRITTENDEN. Three varieties of soybeans (Laredo, Anderson, and Adams) were exposed to *Meloidogyne hapla* for periods of 7, 14, 21, 28, 35, 42, 49, and 56 days. Galls first appeared on Adams at 7 days and on Laredo and Anderson at 14 days. Egg masses were first found on Anderson and Adams at 35 days and on Laredo at 42 days. Studies of osmic-acid–stained roots showed a large number of larvae in the roots of Adams; a lesser number were present in the roots of Laredo and Anderson. In roots invaded by the larvae of *M. hapla*, the growth of the apical meristem often ceased and 2–10 lateral roots arose from the gall. Larvae were found in the apical meristem and in the region of elongation. The larvae were often located at the junction of lateral roots. Measurements of galls showed that most of the hypertrophy occurred in the tissues of the stele. No differences were observed in the giant cells of each of the three varieties. Characteristics of the giant cells were: dense cytoplasm, thick cell walls, and enlarged nuclei.

Studies on certain viruses infecting legumes in California. SCOTT, H. A., AND A. H. GOLD. Four virus collections from field-infected legumes were tested on Perfection and Dwarf Telephone peas, Pinto bean, Persian melon, broadbean, Dutch white clover, and Turkish tobacco. They were found to consist of two strains of bean yellow mosaic virus, veinal necrosis virus, and white clover mosaic virus. The last-named virus infects snapdragon, spinach, mung bean, cowpea, and chickweed, but not cucumber. Its longevity in vitro exceeds 31 days. Attempts to transmit this virus with *Macrosiphum pisi* and *Myzus persicae* and by dodder, *Cuscuta campestris*, were not successful. Apparently the white clover mosaic in this collection is similar to Johnson's pea-mottle–pea-wilt complex but consists of only one component. By using the adjuvant technique of Freund, an antiserum was developed against each virus collected. Only the two strains of bean yellow mosaic were serologically related. Electron-microscope examinations of juice from broadbean infected with each virus showed flexuous rods. The modal lengths of the associated particles were: bean yellow mosaic, 730–750 mμ; veinal necrosis, 650–670 mμ; and white clover mosaic, 530–550 mμ.

The influence of copper and of glyodin on the amino acid and sugar content of Colletotrichum capsici. SIEGEL, M. R., AND D. F. CROSSAN. Spores of *Colletotrichum capsici* were germinated in the presence of a sub-lethal amount of copper phosphate (150 ppm), and of glyodin (6 ppm), which, however, produced a pronounced morphological and physiological effect on the developing mycelia in shake cultures. Paper chromatography was used to evaluate the qualitative and quantitative effect of the 2 fungicides on the free and bound amino acids, and on the sugars, in treated and untreated 76-hour-old thalli. To help substantiate the chromatographic results, the mycelia were subjected to (a) total nitrogen determinations using a

micro-kjeldahl technique, (b) a quantitative analysis of the total amino acids using a modified photometric ninhydrin method, and (c) a Warburg study of respiration. The data obtained indicate that copper caused a general reduction in the amounts of the free amino acids present plus a loss of cystine and arginine; glyodin reduced the amounts of the bound amino acids. Both chemicals reduced the amounts of the sugars, rhamnose, xylose, mannose, and glucose, and caused a loss of a methylated sugar. Warburg studies indicated a drastic curtailment of both exogenous and endogenous respiratory mechanisms in the fungicide-treated mycelia.

Phytotoxicity of captan. SILBER, GUSTAVE. Growth of various plants in both nutrient and soil culture was inhibited by captan. Tomato, Red Kidney bean, corn, and peppermint plants in Hoagland's nutrient solution supplemented with 1, 3, and 10 ppm captan grew less than control plants receiving no captan. Of these plants, tomato was the most sensitive to captan. Fresh and dry weights of tomato plants that received 3 ppm captan were significantly lower (odds of 19:1) than those of the controls. Ten ppm captan reduced the growth of the other plants significantly below that of their respective controls. In a methyl-bromide-fumigated soil supplemented with 0, 25, 100, 200, and 400 ppm captan (dry-weight basis), tomato, Red Kidney bean, corn, cucumber, and cabbage plant growth was inhibited by captan. Tomato growth was significantly reduced by 25 ppm captan, and cabbage growth by 100 ppm. In contrast, corn or bean growth was not significantly affected by less than 400 ppm captan. Slight marginal necrosis of cotyledons and leaves of tomato, cabbage, and cucumber occurred on plants grown in soil containing a sufficiently high concentration of captan to cause stunting.

Vein necrosis, a virus disease of bean. ZAUMEYER, W. J. A new strain of alfalfa mosaic virus that produces primary and systemic necrotic symptoms on beans unlike those produced by the several previously reported alfalfa mosaic viruses was isolated from *Pisum sativum* grown in southern Idaho. This virus differs from all other known strains of virus, including alfalfa yellow mosaic and alfalfa N viruses, the only two that consistently cause systemic necrosis of beans. On Bountiful bean, primary necrotic lesions are not as discrete as those produced by other strains of the virus. Veinal necrosis follows, and large blotched chlorotic areas appear later. Trifoliate leaflets become puckered, but not mottled; and the veins become necrotic. Petioles and stems of severely infected plants are necrotic also, and the terminal growing point is often killed. On Pinto bean, the virus produces local necrotic lesions similar to those produced by the type strain. Veinal necrosis is evident near the primary lesions. The virus seldom becomes systemic in the Pinto variety. The host range of the vein necrosis virus is similar to that of the other strains of the alfalfa mosaic virus. Physical properties conform with those of the type strain. The virus was inactivated in 10 minutes at 62–64°C, and in 32 hours at 18°, and withstood dilution of 1:4000.

Studies on internal bark necrosis of Red Delicious apples. ZEIDERS, K. E., AND H. C. FINK. Internal bark necrosis developed on trees receiving 1) 50 ppm manganese, 2) no boron, or 3) 2, 3, 5, and 50 ppm manganese and no boron, when grown in a modified Hoagland's solution and sand medium. Addition of 250 ppm sodium chloride to the balanced solution did not induce the symptoms. High concentrations of iron seemed to delay but not prevent expression of symptoms induced by manganese toxicity or lack of boron. No macroscopic symptoms resulted from 50 ppm iron applied as "sequestrene (NaFe)." Iron toxicity resulted in rotted roots, terminal rosetting and stunting. Preliminary histological studies indicate necrosis or hyperplasia of parenchymatous cells in all areas of the periderm, cortex, pericycle, or phloem may be the first evidence of either boron deficiency or manganese toxicity in Red Delicious apple trees. The results of the present investigation corroborate previous reports that symptoms of internal bark necrosis are induced by 1) manganese toxicity and 2) boron deficiency. They do not confirm reports that excess iron causes the disease and they indicate that the disease reported on Jonathan as caused by high soil chlorides is distinct and different from internal bark necrosis on Red Delicious.

Rhizoctonia solani inciting a collar rot of tea. K. S. VENKATARAMANI AND C. S. VENKATA RAM. Collar rot of tea seedlings in the nursery is caused by factors that are either pathogenic (soil fungi) or physiogenic (heating of the soil surface and consequent damage to the tender bark).[1, 2] Collar rot is also known to occur in young tea plants in the field, but the attacks are usually sporadic. Recently, however, an epiphytotic of this disease was reported from an estate in Southern India, involving a large number of vegetatively propagated tea plants in an area of about 30 acres. The plants were put out in the field when they were 11–15 months old, and the disease was noticed 8 months later.

Different stages of yellowing of the foliage and withering of the plants were observed. The disease was not confined to any particular clone. Injury at the collar ranged from an initial discoloration and splitting of the bark (Fig. 1-A) to advanced stages of bark disintegration, wound development, and callus formation (Fig. 1-B). Finally, the entire stem was girdled, and a heavy callus growth developed both above and below the injured part. The diseased plants occurred in groups. Field observations ruled out wind and insect damage as causal factors. *Rhizoctonia solani* Kuehn was consistently isolated from plants showing all stages of injury. *R. solani* has been reported to infect tea seedlings in the nursery, but the injury was not confined to the collar region. On the other hand, the fungus was observed to travel up the stem and attack the succulent green shoots and leaves.[3] The present report is the first instance of the fungus causing widespread damage to young tea plants in the field, attacking well developed stems.

R. solani does not usually invade mature bark tissue,[1] and field observations suggested that 2 predisposing factors were responsible for the fungus infection: 1) Deep planting, well below the nursery level (Fig. 1-C), resulted in softening of the bark and rendered it susceptible to fungus entry; 2) injudicious mulching caused injury to the bark at the ground level. Preliminary pathogenicity tests with *R. solani* isolated from the diseased tea plants indicated that the fungus was able to attack two-year-old tea plants in the nursery only when the collar was injured.

The following recommendations are suggested for preventing the disease in new clearings: 1) The young plants should not be planted deeper than the nursery level; 2) when mulching is resorted to, some space is to be left uncovered around the collar of the plant. If the mulching material consists of wood-chips, it should be handled carefully to avoid injury to the collar tissue.—UPASI Tea Experiment Station, Devarshola, S. India.

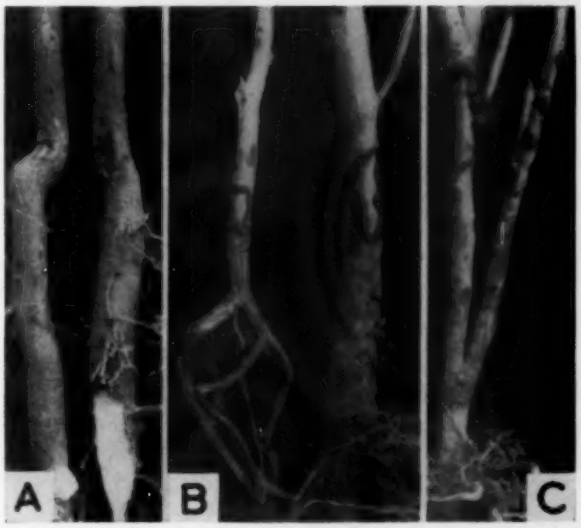

Fig. 1. A) Initial stages of *Rhizoctonia solani* collar rot of tea plants. **B)** Wound development and callus formation seen in advanced stages of infection. **C)** Complete girdling of the stem at the ground level in plants set deep in the soil.

An alcohol agar medium selective for determining Verticillium microsclerotia in soil. MATHEW J. NADAKAVUKAREN AND CHESTER E. HORNER. A rapid method for quantitative determination of microsclerotia-producing strains of *Verticillium albo-atrum* Reinke & Berth.[1] in soil has long been needed. Soil dilution plates with ordinary agar media are not suitable, because rapidly growing fungi soon mask the presence of *Verticillium*. Also, rapidly growing and antagonistic fungi markedly reduce the number of *Verticillium* colonies that can be counted on dilution plates, even when oxgall is used to reduce radial growth of fungi and antibiotics are used to minimize the growth of bacteria and streptomycetes. The technique described by Wilhelm[2] for quantitative determination of the inoculum potential of *Verticillium* in soil requires the use of test plants and a long incubation period. The "most probable number" method described by Maloy and Alexander[3] for estimating populations of plant-pathogenic organisms in soil also requires test plants.

[1] Gadd, C. H. 1949. The commoner diseases of tea. Monographs on Tea Production in Ceylon, No. 2, Tea Research Inst. Ceylon, p. 62-67.

[2] Tunstall, A. C., and K. C. Sarmah. 1947. Notes on stem diseases of Tea in North East India. Indian Tea Assoc. Sci. Dept. Tocklai Exptl. Sta. Mem. 16. p. 54-58.

[3] Gadd, C. H. 1929. Report of the Mycologist. Tea Research Inst. Ceylon Bull. 4. p. 9-12.

[1] *Verticillium albo-atrum* is used in this paper as envisioned by Rudolph, B. A. 1931. Verticillium hadromycosis. Hilgardia 5: 197-353.

[2] Wilhelm, S. 1950. Vertical distribution of *Verticillium albo-atrum* in soil. Phytopathology 40: 368-376.

[3] Maloy, O. C., and M. Alexander. 1958. The "most probable number" method for estimating populations of plant pathogenic organisms in the soil. Phytopathology 48: 126-218.

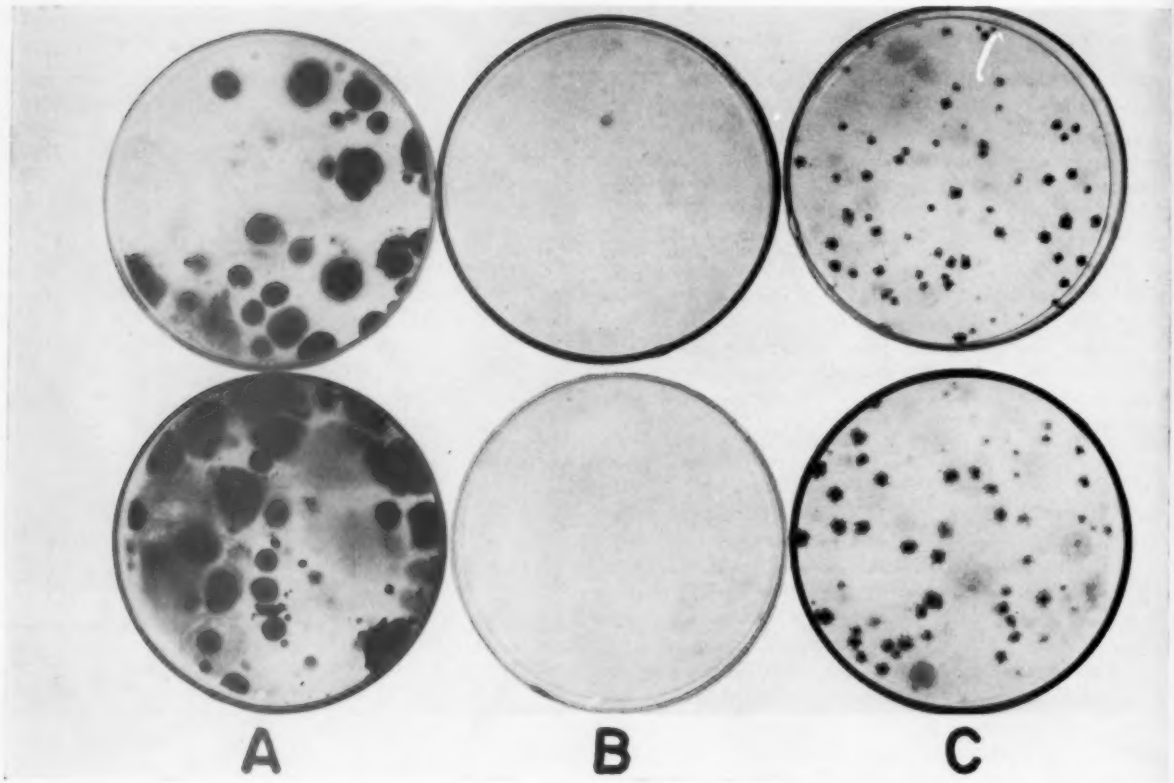

Fig. 1. Comparison of **A)** potato-dextrose agar, **B)** water agar, and **C)** alcohol agar (0.5%) at $1:10^5$ dilution for determining *Verticillium* by soil dilution. All plates were poured from the same sample and photographed after 10 days of incubation. Dark colonies on alcohol agar are *Verticillium*.

The alcohol-agar medium described here has proved selective for determining *Verticillium* on soil dilution plates.

On alcohol agar, *Verticillium* colonies produce microsclerotia, turn black, and can be counted easily against a white background. Most other fungi in our soil samples were colorless and grew sparsely. *Verticillium* colonies can be distinguished macroscopically from other fungi that produce black mycelia.

The successful technique used in our laboratory is as follows. Water agar (7.5 g agar/liter) containing 100 ppm streptomycin is prepared and 90-ml portions are dispensed into 200-ml Erlenmeyer flasks. After being autoclaved the flasks are allowed to cool to 45–50°C, and then maintained at 42–44°C in a constant-temperature bath. Just before the diluted soil samples are mixed with the agar, 0.5 ml absolute ethyl alcohol is added to each flask. After the diluted soil samples are added, the contents of each flask are dispensed into 5 or more Petri dishes and incubated 10 days at 18–23°C in the dark.

In a comparative test, the alcohol-agar medium was superior to potato-dextrose agar and water agar in the accuracy and ease with which colonies of *Verticillium* could be detected and counted (Fig. 1). A single soil sample was used, and all media contained 100 ppm streptomycin. On potato-dextrose agar plates, rapidly growing fungi masked the presence of many Verticillium colonies within 3–5 days. On water agar, *Verticillium* colonies formed microsclerotia sparingly, making determination difficult without microscopic examination. On alcohol agar, *Verticillium* colonies formed abundant microsclerotia in 7–10 days, and colonies stood out sharply in contrast to other fungi when viewed against a white background.

An additional advantage of the alcohol medium is that plates may be held several weeks at room temperature without the accuracy of the determinations being harmed. The chief limitation of the method for field use is that low population of *Verticillium* in a soil sample containing high populations of other fungi cannot be detected unless more medium is used to distribute the fungi in a larger number of dilution plates. However, even if 50 plates were required, the method would still be more efficient than the use of indicator plants.

Alcohol-agar medium has been used successfully, with reproducible results, to determine populations of *Verticillium* microsclerotia in several hundred samples of artificially infested soil. Experiments are being conducted to test the usefulness of this method for quantitative determination of *Verticillium* microsclerotia in naturally infested soil.—Department of Botany and Plant Pathology, Oregon State College, Corvallis, Oregon.

ASSAY OF ALFALFA MOSAIC VIRUS ACTIVITY

Karl D. Fezer and A. F. Ross [1]

In studies of alfalfa mosaic virus (*Marmor medicaginis* Holmes) that were initiated before the recent report of Panzer,[2] reproducible estimates of biological activity of the virus were difficult to obtain. *Phaseolus vulgaris* L. var. Early Golden Cluster, used successfully as a local-lesion test plant by Price and Spencer[3] and by Ross,[4] was no longer available. Therefore, 11 varieties of *P. vulgaris* were screened as test plants for the virus. In addition, 2 methods for obtaining extracts of the virus were compared.

Plants of Turkish tobacco (*Nicotiana tabacum* L.) infected for 11–12 days[5] with AMV (alfalfa mosaic virus, American Type Culture No. 57) were used as the source of inoculum. Both inoculated leaves and leaves in the acute stage of systemic infection proved to be good sources of inoculum. Bean varieties were screened by rubbing the unifoliate leaves of several Carborundum-dusted plants of each variety with a cheesecloth pad saturated with inoculum diluted with 0.01*M* potassium phosphate buffer at pH 7.0. Criteria used in evaluating varieties were lesion number, lesion size and distinctness, and uniformity of lesion size and number. Lesion size was found to depend to a marked extent on the age of the leaf at the time of inoculation; inoculation before leaves were fully expanded resulted in larger lesions than inoculation after full expansion.

In 2 separate tests, Bountiful and Tendergreen proved to be most useful as local-lesion test plants for AMV. Red Kidney and Yellow Eye were also satisfactory. Golden Cluster Wax, Top Crop, Contender, Michelite, Scotia, Pinto, Black Turtle Soup, and Scarlet Runner (*P. coccineus* L.) were all inferior to the above as test plants. Tendergreen has not previously been reported as a suitable test plant for AMV; however, the other 3 varieties herein reported as satisfactory were used successfully by Panzer[2] in assaying AMV.

All test plant varieties were also inoculated with the potato calico strain of AMV (*M. medicaginis* Holmes var. *solani* Black & Price). The varieties ranked as before, but lesions were generally smaller than those due to ATC 57. The tobacco source plants inoculated with the potato calico strain showed symptoms on inoculated leaves only; an extract from leaves immediately above the inoculated ones had very low virus activity.

Ross[5] found that extracts prepared by adding a phosphate buffer to the leaf pulp immediately after grinding had an AMV activity more than twice that of extracts prepared without added phosphate. In the work reported here, adding a buffer solution immediately after grinding was compared with adding the buffer before grinding.

In each of 3 experiments, 2 comparable lots of tissue from systemically infected tobacco leaves were obtained (14–15 days after inoculation of the source plants) by pairing leaf discs according to symptom severity. Enough 0.01*M* potassium phosphate buffer (pH 7.0) was added to give extracts diluted 1:8.5 in a preliminary test and 1:10 in the other 2 tests; the buffer was added to one sample of each pair before the tissue was ground in a mortar, whereas it was added to the other after grinding. For assay, the extracts were diluted still further with 0.01*M* phosphate buffer. In the preliminary test, single dilutions were compared on opposite half-leaves of Bountiful beans. In the other 2 tests, assays were made by a modification of the 2-dilution method of Spencer and Price,[6] as used by Rochow and Ross.[7] The log interval between the 2 dilutions of each extract was 0.5, and that between extracts was 0.5 or 1.0. Inocula were applied to half-leaves in such a manner that each of the 6 possible pairs of the 4 inocula was compared on opposite half-leaves an equal number of times, and each inoculum was applied to the right half as often as it was applied to the left half.

In the preliminary test, a 1:425 dilution of the extract prepared by adding buffer before grinding (Extract 1) induced over half as many lesions as did a 1:42.5 dilution of the extract prepared by adding buffer after grinding (Extract 2). Lesions were so numerous, however, that accurate counts were not possible. In the second test, each inoculum was applied to 24 half-leaves of Tendergreen and 24 half-leaves of Bountiful. The number of lesions induced by the 2 dilutions of Extract 1, which were 1:3160 and 1:10,000, were 76 and 10, respectively; those induced by the 2 dilutions of Extract 2, which were 1:316 and 1:1000, were 242 and 66, respectively. In the third test, each inoculum was applied to 24 half-leaves of Bountiful. Extract 1, diluted 1:3160 and 1:10,000, induced 87 and 6 lesions per half-leaf, respectively; 1:1000 and 1:3160 dilutions of Extract 2 induced 52 and 4 lesions, respectively. Calculations by Sherwood's[8] formula showed that Extract 2 contained slightly over ¼ as much active AMV as did Extract 1(29% in the second test and 26% in the third).

These results suggest that ⅔ to ¾ of the potential alfalfa mosaic virus activity may be lost by the time

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buffer can be added immediately after grinding. Source-plant leaf tissue should therefore be bathed in buffer solution while it is being ground.—Department of Plant Pathology, Cornell University, Ithaca, New York.

LARGE-SCALE SOIL FUMIGATION AGAINST BROOMRAPE

Stephen Wilhelm, R. C. Storkan, James E. Sagen, and T. Carpenter

Methyl bromide applied to the soil is known to control branched broomrape, *Orobanche ramosa* L.,[1, 2] and results of the tests here reported indicate that it also is effective in relatively large-scale field control of Cooper's broomrape, *O. ludoviciana* var. *cooperi* (Gray) G. Beck, a parasite of tomato in the Coachella Valley of California.

In July 1957, six areas, each 100 sq. feet, in a field heavily infested with seed and dried remains of Cooper's broomrape were moistened, covered with polyethylene sheeting, and fumigated in pairs at rates of 0.5, 1.0, and 1.5 pounds of methyl bromide. Exposure was for about 20 hours. Equal quantities of soil from each plot and from unfumigated check plots that had been moistened and covered were transferred to 8-in. pots and planted to tomatoes in greenhouses at Berkeley and Davis, California. No broomrape appeared on tomato plants growing in fumigated soils, but in unfumigated soils broomrape was present after 4 months on 12 of 24 tomato plants, and after 9 months on 45 of 61.

Three hundred and thirty acres in 1957 and 512 acres in 1958 of tomato seed-bed land were fumigated. Not only broomrape, but local weeds, such as desert annuals and Bermuda grass, and root-knot nematodes were also controlled. None of these pests were found in the fumigated acreages, except for heavy infestation of root-knot nematode in a strip about 18 in. wide and a few hundred feet long. This strip was undoubtedly missed by a chisel during injection of the fumigant or not covered by a tarpaulin. Not a single shoot of broomrape was found in the fumigated land. In 1958, following exceptional heavy spring rains, broomrape was abundant on native hosts, especially on *Franseria dumosa* Gray.

Following is a brief description of the method used in fumigating and covering the soil in these large-scale tests. Weedfume[3] was applied at the rate of 260 lb./A. (180 lb. of actual methyl bromide) 8 in. deep through 10 chisels spaced 12 in. apart on the tractor draw bar. The tractor also pulled a spike tooth harrow, to smooth out the furrows made by the chisels, and a smooth, medium-heavy roller. Each chisel was adjusted to deliver about 2.1 ml Weedfume (1.05 ml actual methyl bromide) per linear foot of travel. Pressure for the system was supplied by compressed nitrogen gas. Before the fumigant was injected, one edge of the tarpaulin was buried in a 6-in.-deep, nearly straight-sided furrow made along one of the long sides of the plot. Tarpaulins of polyethylene sheeting, 2 mil thick, 20 feet wide, and up to 1400 feet long, proved satisfactory.

The tractor operator began to fumigate immediately next to the buried edge of the tarpaulin, directing one chisel shank to within 6 in. of the buried edge. As the tractor passed, men stationed along the plot began to pull the tarpaulin over the soil. By the time the tractor made one pass and returned to the starting edge of the field, to fumigate a strip 20 ft. wide, the soil was covered with the tarpaulin. At this instant, a second tractor plowed a furrow the length of the plot parallel to the first furrow and at the line where fumigation stopped so that the edge of the tarpaulin, when taut, extended just to the bottom of the second furrow. In making this furrow, the soil was thrown out, away from the plot. It is of great importance that this second furrow be made parallel to the first. The imprint of the wheel of the tractor used for fumigation serves as a guide for this furrow.

The tarpaulin is now pulled taut over the soil and held in place in the furrow by shovelfulls of soil placed at about 3-ft. intervals along the edge of the tarpaulin. Any rents in the covering are patched, and the furrow is closed with a plow. The short ends of the tarpaulin are buried by hand.

The next day the fumigation process is repeated in an adjacent strip, and in covering the newly fumigated strip the tarpaulin is turned over. The edge of the tarpaulin buried before fumigation commenced the day before is removed from the soil, and the tarpaulin gathered into tucks along the edge left buried. As the newly fumigated area is covered, the tarpaulin is turned over. Using tarpaulins 20 feet wide and 1400 feet long and 12–14 men to turn it, an area slightly greater than half an acre was fumigated in 15–20 minutes.

As in soil fumigation with chloropicrin,[4] the soil must be at a proper moisture for seed germination and well prepared as to tilth. In the fumigation of sandy soil, described above, the soil was leveled, manured, and sprinkled before fumigation.

It is believed that fumigation followed by covering of the soil as described offers great promise for eradicating broomrape at minimum doses of fumigant and at low cost. The greatly improved stand of tomato plants resulted in considerable savings of seed, their improved and uniform growth made cultivation and harvesting easier, and the elimination of weeds together with the control of root knot nematode com-

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[4] Wilhelm, Stephen, and R. C. Storkan. 1958. Soil fumigation with chloropicrin. Western Fruit Grower 12: 11-13.

Fig. 1. Immediately after Weedfume injection, polyethylene sheeting is pulled across the treated area. The edges are sealed by burying in deep furrows.

pletely offset the cost of fumigation.—Department of Plant Pathology, University of California, Berkeley 4, California; Neil A. Maclean Company, Belmont, California; and California State Department of Agriculture, Riverside, California.

THE UPTAKE OF VITAL DYES BY THE MYCELIUM OF WHEAT STEM RUST IN SITU[1]

William Silverman[2]

Chlorosis and/or necrosis of host cells is associated with the different resistant reactions of various wheat varieties to certain races of the stem rust fungus (*Puccinia graminis* Pers. var. *tritici* Eriks. & E. Henn.). In the reaction characterized by the rust infection type 2, the necrosis occurs in the form of a ring encircling a green island, in which the mycelium of the fungus sporulates.[3] The necrosis of the infection type 2 lesion is confined to the periphery of the mycelial area, unlike the necrosis found in the infection type 0; or infection type 1 lesions, where the necrosis is generalized over the whole mycelial area. Through the use of vital dyes, the infection type 2 formed by stem rust race 38 on Marquis wheat was studied to seek an explanation of the formation of a peripheral ring of necrosis as opposed to a generalized necrosis.

During the early phases of rust development, before sporulation begins, Marquis wheat and race 38 of the stem rust fungus exist in apparent congeniality. Studies on killed and fixed material stained with Flemming's triple combination modified by a fast green counter-stain revealed, however, that the rust mycelium in the host did not stain uniformly, that is, the older portions of the mycelium stained with fast green but the younger parts, e.g., the hyphal tips and the recently formed haustoria and haustorium mother cells, stained with orange-G.[4] Since orange-G is a cytoplasmic stain, one may infer that the younger portions of the rust mycelium contained a more dense cytoplasm than the vacuolated older portion of the mycelium. The greater concentration of cytoplasm at the tips of the hyphae and in the most recently formed haustoria and haustorium mother cells suggested that metabolic activity, which is associated with dense cytoplasm, was probably greater in these regions than in the vacuolated hyphae found in the center of the infection. The absorption of materials from the host and the secretion of substances by the rust would presumably occur in those regions of the rust mycelium that are metabolically most active. By exposing the rust mycelium growing in the host to a solution of a vital dye, it was thought that the region of maximal metabolic activity would be revealed by a differential uptake of the dye by the rust hyphae.

For this purpose, 2 vital dyes were selected: methylene blue (a 1:1000 aqueous solution) and neutral red (a 0.5% aqueous solution made slightly alkaline with potassium hydroxide). The epidermis covering developing uredia was stripped off, and a drop of the dye solution was placed over the parenchyma in which the rust mycelium was growing. The dye solution was allowed to remain in contact for a few seconds, and then was removed by washing with water. The treated leaves were examined microscopically by transmitted and/or reflected light.

The results obtained with the vital dyes confirmed the findings made with the modified Flemming combination, i.e., that marked staining differences exist between the younger and older portions of the rust mycelium. Since the results for both neutral red and methylene blue were similar, the following description applies to both. It was found that very rapid absorption of the dye occurred at the hyphal tips and at the younger haustoria and haustorium mother cells. Uptake of the dye was almost instantaneous at these sites, and the intensity of coloration indicated that large quantities were absorbed. However, the older portion of the rust mycelium, that portion which stained with fast green in the Flemming combination, took up the dye much more slowly, often requiring as much as 5 minutes before a slight dye color in the hyphae was noted; the intensity of color in the older mycelium never approached that found in the younger portions of the same mycelium. The lack of uptake of the dye by the mycelium in the center of the lesion could not be attributed to inability of the dye solution to penetrate the tightly packed mycelium, for it was observed that host cells in the same region readily absorbed the dye.

The results indicate that the absorption of water-soluble dyes, and, therefore, possibly of other water-soluble substances, e.g., metabolites, occurs most

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rapidly and primarily in the hyphal tips and in the most recently produced haustoria and haustorium mother cells; the older mycelium is relatively inactive in the uptake of the dye and, possibly, other water-soluble substances. Also, the portion of the mycelium that is metabolically most active, whether in absorption or secretion, is in the peripheral region of the lesion, that region in which the necrotic ring develops. If necrosis of host cells is associated with metabolic activity of the rust parasite, as seems very likely, then the restriction of the metabolic activity of the rust to the most recently formed hyphal tips, haustoria, and haustorium mother cells would occasion the development of a peripheral necrotic ring, characteristic of infection type 2, instead of the generalized necrosis found in other resistant reactions. Since it has been determined[4] that no necrosis appears in the developing lesion until the rust mycelium begins to form spores, it appears that sporulation is the triggering mechanism that upsets the benign relationship existing between rust and host previous to sporulation and therefore initiates the processes culminating in the necrosis of host cells.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul 1.

BOOK LISTING

SOUTHEY, J. F. ed. 1959. **Plant nematology.** Ministry of Agriculture, Fisheries, and Food (England) Tech. Bull. No. 7, 175 p. Published by Her Majesty's Stationery Office, London. Price 9s. 6d.

This publication is based on 21 lectures given in 1956 as part of a special training course in nematology at the National Agricultural Advisory Service Regional Headquarters, Bristol, England. It contains six sections: The Nematoda, the principal genera of plant-parasitic nematodes, the importance of cyst-forming eelworms in agriculture, research problems with *Heterodera* species, control, and laboratory culture and nutrition studies.